University of Alberta

Natural Attenuation at Upstream Oil and Gas Sites in Western Canada

by

Katharine Mary Cross ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of **Master of Science**

in

Environmental Science

Department of Civil and Environmental Engineering

Edmonton, Alberta

Spring, 2002

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ABSTRACT

Analysis of groundwater geochemical data for evidence of natural attenuation (NA) has been well documented for processed (downstream) fuel contaminated sites. This study undertakes a similar approach, by reviewing and categorizing relevant case records at sites associated with upstream oil and gas production. A database was constructed to evaluate the occurrence and extent of natural attenuation from existing field monitoring data. Over 200 sites were analyzed for groundwater and plume characteristics. Results indicate desirable characteristics and conditions for successful NA implementation at upstream sites. A case study was also conducted at an upstream diesel contaminated site. Evidence of intrinsic bioremediation included decreasing contaminant concentrations over time, depletion of terminal electron acceptors and increases in microbial metabolic by-products. A laboratory microcosm study investigated the effects of temperature, electron acceptor and nutrient amendment under anaerobic and aerobic conditions. Results indicated that the groundwater was severely nutrient depleted.

Acknowledgements

First, I would like to thank Dr. Kevin Biggar for providing me with the opportunity to take part in this project, giving me excellent guidance and answering my numerous questions. I would also like to thank Dr. Julia Foght and Dr. Selma Guigard for all their advice, input and direction throughout my thesis. I would like to acknowledge the microbiology group for providing the necessary laboratory area and equipment. I would especially like to thank Kathy Semple for aiding with the design and set up of the microbiological experiments, also Sara Ebert, Debbie Coy and Ruth Eckford for assisting me in difficult situations and answering my questions.

I thank James Armstrong from Komex International Ltd. His boundless enthusiasm and willingness to explore different ideas was greatly appreciated. James was a significant contributor to the Plume-a-thon portion of this thesis. I would also like to thank the staff at Komex International for providing office and fieldwork assistance and giving me the opportunity to work at an inspiring company.

The site investigation was carried out with the help of Komex, Dr. Biggar and Christine Hereygers, Gerry Cyre and Steve Gamble. I thank them for their patience and indispensable assistance.

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LIST OF NOMENCLATURE AND ABBREVIATIONS

ATP - Adenosine Triphosphate

ASTM – American Society for Testing and Materials

BART™ - Biological Activity Reaction Test

BEG – Bureau of Economic Geology

BTEX - Benzene, Toluene, Ethyl Benzene and Xylenes

CDWG – Canadian Drinking Water Guidelines

CORONA - Consortium for Research on Natural Attenuation

DCM - Dichloromethane

DO - Dissolved Oxygen

DoD - Department of Defense (US)

DIPA - Diisopropanolamine

DN - Denitrifiers

EBC - Expressed Biodegradation Capacity

Eh° - Redox Potential

ΔG - Gibbs free energy of a reaction

G_f – Gibbs free energy of formation of a compound

ΔH - Enthalpy of a reaction

H_f – Enthalpy of formation of a compound

HGDB - Hydrogeological Database

IRB - Iron Reducing Bacteria

Komex - Komex International Ltd.

LNAPL - Light non-aqueous phase-liquid

LUFT – Leaking Underground Fuel Tank

LUST – Leaking Underground Storage Tank

MNA - Monitored Natural Attenuation

MPN - Most Probable Number

N - Nitrogen

NA – Natural Attenuation

Non-PHC - Non-Petroleum hydrocarbon

NAPL - Non-aqueous phase liquid

P - Phosphorous

PAH - Polynuclear aromatic hydrocarbon

PHC - Petroleum hydrocarbon

RBCA – Risk Based Corrective Action

SRB - Sulfate Reducing Bacteria

STP - Standard temperature and pressure

Sulfolane – tetrahydrothiophene sulfolane

SVE - Soil Vapour Extraction

TCA - Tricarboxylic Acid Cycle

TEA - Terminal Electron Acceptor

TEAP - Terminal Electron Accepting Process

TEH - Total Extractable Hydrocarbons

TPH: Total Purgeable Hydrocarbons

US EPA – United States Environmental Protection Agency

VOC: Volatile Organic Compound

1. INTRODUCTION

Natural attenuation (NA), also known as intrinsic or passive remediation, has recently emerged as a remediation approach used to clean up contaminated groundwater sites (Wiedemeier et al., 1999). Monitored natural attenuation (MNA) refers to the process of documenting a reduction in contaminant mass and concentration due to natural processes when the source has been removed (Wiedemeier et al., 1999). The reduction may be due to a combination of physical and chemical processes (advection, dilution, dispersion, sorption, volatilization, and chemical transformations), and biological processes (biodegradation by indigenous organisms) (Wiedemeier et al., 1999). Of these, biodegradation (intrinsic bioremediation) and chemical transformations are the only mechanisms that will destroy contaminant mass.

Natural attenuation is not a "do-nothing" approach to site clean up. A careful study of the site conditions is necessary to estimate the effectiveness of natural processes in reducing contaminant concentrations over time. Data collection and analysis should be used in conjunction with risk assessment to verify the effectiveness of natural attenuation before it is implemented as a remediation alternative (Ellis and Gorder, 1997). If natural attenuation is the preferred remediation option, a monitoring plan must be implemented and responsibility taken for long-term monitoring. This is to ensure that the contaminants in the groundwater do not pose a potential risk to humans and the surrounding environment (US EPA et al., 2000).

This study focuses on the use of NA at upstream oil and gas sites. Oil and natural gas are extracted from the ground at upstream sites (*e.g.* production wells) and transported to downstream sites (*e.g.* refineries). Preparation for transport involves removing water, carbon dioxide and hydrogen sulfide from oil and natural gas. The unrefined product is transported downstream to a refinery where it is separated into different grades of hydrocarbons (*i.e.* lighter and heavier hydrocarbons). These grades are blended to form the final products, such as gasoline or diesel. Upstream extraction and treatment processes have the potential to contaminate the surrounding environment. Spills and leaks may result in unrefined hydrocarbons and process chemicals seeping into the groundwater. Contamination at upstream sites has not been well documented in the literature. This study aims to address some of the complexities of upstream sites, including the presence of co-contaminants, potentially more complex organics and finer grained soils.

1.1 PETROLEUM HYDROCARBON CONTAMINATION

In Canada, almost 8 million people (~26% of population) rely on groundwater for domestic use. In addition, approximately 27% of Albertans (mostly in rural areas) use groundwater as their main water source (Environment Canada, 2001). Contamination of groundwater may occur from the release of petroleum hydrocarbons (PHCs) into the subsurface, which can impact residential water wells. Sources of PHC releases include storage tanks, pipes, wastewater ponds, wells and other plant facilities. PHC contaminants can pose health and environmental risks to potential receptors near the site of contamination.

Specific components of PHCs can be hazardous to human health. Benzene, a soluble compound commonly found in hydrocarbon mixtures, and can cause bone marrow damage, leukaemia, blood disorders, upper respiratory tract infections and cancer. Toluene is another soluble hydrocarbon of concern that can irritate skin and eyes, and cause kidney and liver damage (Covello and Merkhofer, 1993). Therefore, exposure to these contaminants via contaminated groundwater is a potential risk that must be considered when planning site remediation.

1.1.1 CONTAMINANT PROPERTIES AND BEHAVIOUR

Fuel hydrocarbons occur in a variety of refined forms such as diesel, gasoline, jet fuel (*e.g.* JP-5, JP-4), kerosene and fuel oil. The chemical composition of these petroleum products consists of a mixture of organic compounds. Table 1-1 and Table 1-2 list the components of gasoline and diesel. The properties of PHCs are an important factor affecting the amount of contamination that dissolves in the groundwater. Some components of petroleum fuels will dissolve into groundwater more readily. For example, BTEX compounds (benzene, toluene, ethylbenzene, and total xylenes) have a relatively high solubility in comparison to alkanes such as dodecane. Table 1-3 lists the physical and chemical properties of some of the components of diesel fuel.

Table 1-1. Composition of Gasoline

Compound or Class	Concentration (% Composition)[1]	Concentration (% Composition)[2]
Alkanes	10.8 – 24.3	46.5
Branched Alkanes	17.9 – 57.1	
Cycloalkanes	0.9 – 2.5	
Straight Chain Alkenes	2.3 – 3.5	9.3
Branched Alkenes	3.3 – 4.1	
Cycloalkenes	0.2 – 0.2	
Benzene	0.1 – 3.5	2.0
Toluene	2.7 – 21.8	20.3
Ethylbenzene	0.4 – 2.9	0.9
Total Xylenes	3.2 – 8.3	4.2
Total Aromatics	10.8 – 55.9	44.2
Naphthalene	0.1 – 0.5	

[1] Riser-Roberts (1992)

[2] Fetter (1999)

Table 1-2. Organic Compounds in Diesel Fuel #2 from Riser-Roberts (1992)

Component	Type	Concentration (% Volume)
C_{10}	Alkanes	0.9
	Cycloalkanes	0.6
	Aromatics	0.4
C_{11}	Alkanes	2.3
	Cycloalkanes	1.7
	Aromatics	1.0
C_{12}	Alkanes	3.8
	Cycloalkanes	2.8
	Aromatics	1.6
C_{13}	Alkanes	6.4
	Cycloalkanes	4.8
	Aromatics	2.8
C_{14}	Alkanes	8.8
	Cycloalkanes	6.6
	Aromatics	3.8

Table 1-2 continued

Component	Type	Concentration (% Volume)
C_{15}	Alkanes	7.4
	Cycloalkanes	5.5
	Aromatics	3.2
C_{16}	Alkanes	5.8
	Cycloalkanes	4.4
	Aromatics	2.5
C_{17}	Alkanes	5.5
	Cycloalkanes	4.1
	Aromatics	2.4
C_{18}	Alkanes	4.3
	Cycloalkanes	3.2
	Aromatics	1.8
C_{19}	Alkanes	0.7
	Cycloalkanes	0.6
	Aromatics	0.3

Table 1-3. Physical-Chemical Properties of Selected Components of Diesel (Eastcott et al., 1988)

Compound	Vapour Pressure (kPa)	Solubility (g/m^3)	Henry's Law Constant (unitless)	Log K_{ow}
Benzene	12.7	1790	0.224	2.13
Toluene	3.8	579	0.244	2.65
Ethylbenzene	1.27	135	0.404	3.13
p-xylene	1.17	221	0.227	3.18
n-xylene	1.10	160	0.294	3.20
o-xylene	0.882	215	0.176	3.13
1,3,5 Trimethylbenzene	0.328	48.2	0.330	3.55
1,2,4 Trimethylbenzene	0.271	51.9	0.253	3.58
1,2,3 Trimethylbenzene	0.202	65.5	0.15	3.58
Hexane	20.2	12.3	57.1	4.11
Dodecane	0.0157	0.0034	317	
Undecane	0.0522	0.044	74.9	
Naphthalene	0.0111	30.6	0.0562	3.35
2-Methylnaphthalene	0.00903	25.6	0.0203	3.86
Fluorene	8.00×10^{-5}	1.84	0.00356	4.18
Phenanthrene	1.61×10^{-5}	1.18	0.00145	4.57

When PHC contamination enters the subsurface it either becomes dissolved in water, sorbed onto matrix particles, volatilized in soil vapours or remains as non-aqueous phase liquid (NAPL). Fuel hydrocarbon NAPL consists of organic hydrophobic liquids that are less dense than water and tend to float on top of the water table (Wiedemeier et al., 1999; Parsons Engineering Science, 1999b). This form of NAPL is referred to as light non-aqueous phase liquid (LNAPL).

Petroleum hydrocarbon groundwater plumes can be formed under a variety of scenarios. First, water can be contaminated at the groundwater surface by a leaking surface impoundment (*i.e.* pond) or an injection well. Contaminants in this scenario are mainly in the dissolved phase. Secondly, LNAPL can be introduced into the vadose zone (area between ground surface and water table) and remain in this area as residual LNAPL. Rainfall or other water discharge infiltrates the subsurface and encounters residual LNAPL, where contaminant compounds in the residual NAPL dissolve and form leachate that may migrate into the water table. LNAPL may also penetrate the vadose zone, pooling atop the water table. This LNAPL provides a continuous source of dissolved contaminants to the groundwater (Wiedemeier et al., 1999).

1.1.2 REMEDIATION OPTIONS

Contaminant removal is dependent on characteristics of contaminants, site geology and hydrogeology, as well as remediation technology (Suthersan, 1997). In many cases removal of the source of contamination will speed up remediation. Excavation and treatment of contaminated soil is known as *ex-situ* treatment (Downey et al., 1999). Remediation technologies associated with *ex-situ* treatment include landfarming, biopiles, off-site disposal and thermal desorption. These technologies are applicable at sites shallow enough to permit excavation where contaminated soil poses a high risk to the surrounding environment. However, there is the increased risk to potential receptors during handling and transportation. It is often more cost-effective and safer to conduct *in situ* remediation.

In situ remediation takes place without removing contaminated media from the subsurface. *In situ* technologies include pump and treat, bioventing, soil vapour extraction (SVE), air sparging, biological enhancement, the use of permeable reactive barriers and numerous other developing technologies.

Natural attenuation is also classed as an *in situ* remediation option that can be used both as a supplement to engineered treatment systems, and as a cost-effective alternative engineered remediation. Combining engineered remediation and natural attenuation may rapidly reduce contaminant concentrations within the source area (Downey et al., 1999). At many sites, natural attenuation is the only appropriate treatment, regardless of costs. Natural attenuation is considered as the first remediation option to be evaluated at US Air Force sites (Kennedy et al., 2001). However, engineered remediation may be required to achieve clean-up goals in a specified amount of time (Downey et al., 1999).

There is increasing evidence that at most PHC contaminated sites natural attenuation may be effective at mitigating long-term risks to human health and the environment. Natural attenuation may be implemented to remediate the remainder of the site once the source has been removed, and residual contamination that may remain after engineered remediation has been completed.

1.2 ADVANTAGES AND LIMITATIONS OF NATURAL ATTENUATION

Natural attenuation has a number of advantages over conventional engineered remediation. Intrinsic bioremediation of PHCs ultimately converts contaminants into innocuous by-products, such as carbon dioxide and water (Wiedemeier et al., 1999). Contaminants are destroyed partially or completely rather than transferred from one environmental medium to another (Van Cauwenberghe and Roote, 1998). Natural attenuation is nonintrusive, does not interfere with ongoing site operations, and there is no future liability with hauling and waste disposal. There are also no limitations due to mechanized equipment (Wiedemeier et al., 1995a). Remediation of contaminants takes place *in situ*, thus the potential for exposing site workers to hazardous materials is reduced (NFESC Environmental Department, 2000).

Natural attenuation is often the remediation technology of choice due to the low cost in comparison to other engineered approaches. Parsons Engineering Science (1999a) conducted a cost analysis for 35 US Air Force sites. The average proposed long-term monitoring program had an estimated cost of $192,000 per site over 30 years with a network of 11 monitoring wells. On the other hand, engineered source removal (bioventing or SVE) was predicted to cost an average of $591,000 and reduce monitoring to a period of 20 years. A combination of natural attenuation and engineered remediation would reduce site cleanup to an average of 15 years but cost an average of $816,000 per site. It should be noted that some form of engineered source removal was recommended at 66% of the US Air Force sites approved for NA (Parsons Engineering Science, 1999a).

Downey et al. (1999) also estimated potential costs of natural attenuation compared with other remediation technologies at a hypothetical fuel spill site. The total cost of monitored natural attenuation over 20 years would be $290,000. The cost of air sparging was estimated at $277,000 over 3 years, however there would also be a large start up expense. Also, pilot tests would have to be conducted during air sparging, which would add to the cost. Pump and treat costs would amount to $1,550,000 over a period of 10 years. During 1996, the US Department of Defence (DoD) operated 75 pump and treat systems to remediate contaminated sites. This technology remediated contamination slowly, cost more than $500,000 per site and did not allow DoD to meet the required clean-up goals within a reasonable time (US DoD, 1998). It is evident that natural attenuation may be a cost-effective option that can result in reliable remediation of a site. Thus, when considering various options for remediation, it is advantageous to first determine whether the site can be remediated naturally. Consequently, site characterization is conducted to support the choice of natural attenuation as a remediation option. In addition, there is a very small incremental cost to study natural attenuation viability when site characterization is being carried out.

Natural attenuation has several potential limitations including long remediation time frames due to potentially slow biodegradation rates. A site may not be cleaned up to regulatory standards if there is regulatory or economic pressure to remediate in a short time period. Furthermore, responsibility must be assumed for long-term monitoring and the associated costs (Wiedemeier et al., 1999). Natural attenuation may not be effective if a plume intersects an environmental receptor such as residential water wells or wetlands. In such cases, more aggressive engineered remediation may be necessary. Natural and anthropogenic changes in hydrogeological conditions as well as aquifer heterogeneity may complicate site characterization (Wiedemeier et al., 1995a). Hydrologic and geochemical conditions that are amenable to NA may change over time and result in renewed mobility of previously stabilized contaminants, such as manganese and arsenic (Wiedemeier et al., 1999). In this case and when the contaminants are recalcitrant, NA may not be an appropriate stand-alone remediation option (NFESC Environmental Department, 2000).

1.3 SITE CONDITIONS

For natural attenuation to be successful at a site, the hydrogeology, geochemistry and contaminant properties must be taken into consideration. Several characteristics must be present at a site for successful intrinsic bioremediation. These include an area where there is minimal risk of contaminants coming into contact with receptors and the presence of adequate microorganisms, nutrients and terminal electron acceptors (TEAs) (Van Cauwenberghe and Roote, 1998; Johns ,1999). Even if a site does not meet all these criteria, a bioremediation strategy can be developed for the non-ideal site.

In addition, certain microbiological, geochemical and hydrogeological conditions must be met. Hydrocarbon-degrading organisms need to be present, but they are rarely a limitation. There are numerous microorganisms that will degrade PHCs and they are widespread in the environment and occur in fresh and salt water, soil and groundwater (Borden et al., 1995). There should be an adequate supply of readily available electron acceptors to satisfy the biodegradation capacity of a plume (detailed in Section 3.2). A low concentration of toxic substances is necessary, because compounds such as lead, arsenic, or even salinity may be inhibitory to biodegradation. The pH and temperature must be adequate for biodegradation to occur. Neutral or near neutral pH values are optimum for biodegradation. Temperature effects on biodegradation can be complex; usually the rate of enzymatic degradation and bacterial metabolism doubles for every 10^0C increase (Sawyer et al., 1994). Nonetheless, several studies have found that intrinsic biodegradation occurred despite cold (4-10°C) *in situ* temperatures (Tumeo and Guinn, 1997; Whyte and Greer, 1999; Billowits et al.,1999; Johns, 1999; Margesin and Schinner, 1999; Braddock et al., 2001).

The effectiveness of NA at various sites has been investigated in areas with a wide variety of environmental and contaminant conditions. For example, the Air Force Centre for Environmental Excellence (AFCEE) has looked at sites ranging from Alaska to Florida, with groundwater depths up to 14 metres below the surface, plume areas from 0.12 to 24 hectares, groundwater temperatures ranging from 5.5 to 26.9 °C, and silty clay to coarse sand and gravel soil types (Parsons Engineering Science, 1999a). To date, formal regulatory acceptance of NA has been obtained for approximately 17 of the 42 sites, including sites in Alaska, Texas, South Carolina, Arkansas, South Dakota, Washington, Virginia, Montana, Florida, New York, Ohio, Massachusetts and Michigan (Parsons Engineering Science, 1999a).

1.4 THESIS OVERVIEW

This thesis examines the viability of NA as a remediation option at upstream oil and gas sites, in the form of a database, then focuses on natural attenuation at a specific site. Data were compiled from potential NA sites assessed by Komex International Ltd (Komex). Analysis and interpretation of these data produced tentative conclusions to assess NA at contaminated sites. Further details are provided in the program objectives (Section 2.1).

The investigation of NA is multidisciplinary. Knowledge from microbiology, geology, geochemistry and hydrogeology are used to assess each site and analyze data, as discussed in Section 3. Methodology, results and discussion of the plume-a-thon database are given in Section 4. The case study of NA in Sections 5 and 6 includes field and laboratory investigations. Section 7 discusses limitation and future considerations for further study. Section 8 summarizes the main conclusions.

2. PROGRAM OBJECTIVES AND SCOPE

2.1 OBJECTIVES

This study evaluates the effectiveness of NA to remediate contaminants at upstream oil and gas sites in western Canada. The first objective was to identify winning and losing conditions where NA might provide a reasonable remediation approach. If successful, this identification could be applied to monitoring programs at other sites with less documentation. This process involved generating and analyzing a plume database with more than 200 plumes ("a plume-a-thon"). These plumes were examined and categorized according to monitoring data, site details and plume characteristics. Analysis of the database involved correlating evidence of NA to site and plume characteristics.

The second objective was to evaluate intrinsic bioremediation of diesel fuel, in particular total extractable hydrocarbons (TEH) range organics, at a site in Central Alberta. TEH compounds consist of heavier hydrocarbons in the ranges of C_{10}-C_{30}. The majority of published natural attenuation research has focused on the natural attenuation of BTEX compounds and little emphasis has been made on TEH compounds. Field data and laboratory experiments were used to determine the anticipated rate and extent of TEH mineralization to be expected under *in situ* conditions, and the potential effects of electron acceptor and nutrient amendment to enhance the process. The effect of temperature on intrinsic bioremediation of diesel was also explored.

2.2 THREE LINES OF EVIDENCE TO SUPPORT NA

Site-specific characterization data and analysis are required to support the implementation of NA. There must be sufficient evidence that contaminant degradation is occurring at a rate that will prevent further migration of the contaminants to potential environmental acceptors. Additionally, a reasonable time length to reach clean-up objectives must be achieved (Armstrong et al., 2000).

Wiedemeier et al. (1995a) developed a technical protocol for data collection and analysis in support of intrinsic bioremediation with long-term monitoring for restoration of groundwater contaminated with fuel hydrocarbons. The protocol explains and shows how to implement three "lines of evidence" to determine that degradation of site contaminants is occurring at a rate sufficient to be protective of human health and the environment. The American Society for Testing and Materials (ASTM) has a standard guide to determine whether natural attenuation is an appropriate form or remediation. This guide also contains implementation guidelines for NA at a petroleum contaminated site as a stand-alone process or with other remediation technologies (ASTM, 1998). In addition, natural attenuation protocols have been developed by the US EPA, US Department of Energy, US Air Force, US Navy, 10 US States, Chevron, American Petroleum Institute, and Remediation Technologies Forum (National Research Council, 2000; Epp, 2000).

The first line of evidence is provided by historical groundwater and/or soil chemistry data that show reductions or stabilization in plume geometry and contaminant mass (or concentration). The second line of evidence involves recording relative changes (between plumes and background areas) of geochemical indicators associated with terminal electron accepting processes (TEAPs). This includes measuring the depletion of electron acceptors (dissolved oxygen, nitrate and sulfate), enrichment of metabolic by-products (manganese (II), iron (II) and methane) and presence of biodegradation metabolites or intermediates.

The third line of evidence is direct microbiological data from field or microcosm studies. These data are often optional but will directly demonstrate that indigenous microorganisms can degrade contaminants under site conditions. Furthermore, a laboratory rate of degradation can be calculated, and the dominant electron accepting process can be identified.

2.3 PLUME-A-THON

Much of the impetus behind the rapid acceptance of natural attenuation has derived from database studies examining downstream oil and gas contaminated sites (summarized in Section 3.8). These programs were originally conducted to look for 'typical' responses, and assess if correlations with 'characteristic' site factors could be drawn from the compiled data. A similar approach was taken in this study to examine monitoring data compiled for a variety of upstream oil and gas production and handling facilities in Western Canada. This project is part of a larger program identified as Consortium for Research on Natural Attenuation, hence is called the CORONA 'plume-a-thon' database. The project was developed in association with Komex.

Research on NA trends at upstream oil and gas sites has been minimal. The majority of studies focus on contamination at downstream facilities. Thus, the objective of the CORONA 'plume-a-thon' was to generate and analyze information in a database of monitoring and site characterization data specifically from upstream sites. The analysis focuses on characterizing typical plumes at upstream facilities and correlating evidence of NA to site and plume characteristics. Information about each site and plume was derived from Komex reports. Specific plume and site characteristics were identified using methods described in Section 4.2.

Figure 2-1 shows the spatial distribution of Alberta sites included in the database; data from all sites (including British Columbia, Saskatchewan and the Northwest Territories) were included in the trend assessments. The majority of sites included in this study contained PHC contamination. A few sites with only inorganic and process chemical contamination were included for comparison.

Historical data and geochemical indicators were the two lines of evidence commonly assessed and gathered during site investigations. Analysis of the CORONA database focused on these lines of evidence to determine the effectiveness of using NA at a site.

The aim of the CORONA database was to identify trends that will help determine the viability of NA at upstream oil and gas sites. This was followed by a case study at an upstream oil site. The site is contaminated with diesel fuel from an invert drilling mud spill, which has migrated into the underlying bedrock.

2.4 CASE STUDY

The study site was investigated to evaluate the viability of NA of TEH range PHCs as a remediation option. A map of the site is shown in Figure 2-2. Subsurface contamination occurred on November 30th, 1982 when an unknown quantity of invert mud discharged from the well, mud tanks and flare line onto the adjacent land. The invert mud contained diesel fuel, which subsequently seeped into the groundwater at a depth of approximately 30 m in fractured bedrock. A clean-up program was started immediately between December 1st and 8th, 1982. The initial groundwater investigation was implemented in 1996. Due to the extreme difficulty of remediation of fractured bedrock at such a significant depth, the viability and extent of natural attenuation is of interest.

In 1997 the drill sump was excavated and impacted soil was stockpiled on the well lease site. In 1998 the soil was remediated by screening and separating fine-grained material from coarse. The fine-grained materials were taken to a Class II landfill, and the coarse material was used as backfill. Soil vapour extraction (SVE) and multiphase extraction (MPE) were carried out as test pilot projects in 2000. Free-product was also bailed out of a few wells with a PetroTrap passive bailer system. Both of these measures have had limited success. Excavation of the contaminated drilling mud from the sump on the east side of the well lease was the main remediation strategy to date that could be termed successful (Brown, 2001).

Monitoring wells were installed in 1996, 1998, 2000 and 2001 to regularly sample the groundwater for contaminant and geochemical parameters. In addition, groundwater was collected to conduct a series of microbial experiments to determine population and activity of the indigenous microorganisms. Historical, geochemical and microbial evidence was used in this study to assess the effectiveness of NA to remove the diesel contamination.

2.4.1 HISTORICAL EVIDENCE

Historical evidence includes groundwater and/or soil chemistry that demonstrates a clear decreasing trend of contaminant mass and/or concentration over time (Wiedemeier et al., 1999). Komex had gathered data from the study site since 1996. These data were examined for decreasing, increasing and stable trends in both time and space. There has been concern that the diesel plume may potentially be migrating further downstream; thus additional monitoring wells have been installed over the past five years.

2.4.2 GEOCHEMICAL EVIDENCE

Geochemical data were used to determine the types of intrinsic bioremediation processes active within a plume. Additionally, the rates of biological degradation processes were also quantified (Wiedemeier et al., 1999).

Biodegradation of PHCs results in detectable changes in the chemistry of the contaminated groundwater. Measurement of geochemical changes can aid in documenting and quantitatively evaluating NA at a site (Wiedemeier et al., 1995a). Geochemical data were gathered at the study site to confirm whether biological processes were actively degrading the hydrocarbon contamination, and to determine the dominant TEAP. Furthermore, the potential capacity of the groundwater to biodegrade the dissolved hydrocarbons was calculated, which helps decide whether NA is an effective solution to remediate the site.

Hydrogeological data from previous sampling events were also examined in conjunction with geochemical data to assess the role of other natural attenuation processes. This includes hydraulic conductivity, groundwater velocity and hydraulic gradient. Other data that may potentially be important for site characterization include groundwater pH, temperature and conductivity. The pH of groundwater affects the activity of microorganisms. Hydrocarbon degrading microorganisms prefer a pH of 6-8. Temperature affects metabolic activity of microbes. Low groundwater temperature may greatly reduce biodegradation rates (Wiedemeier et al., 1995a).

2.4.3 MICROBIAL EVIDENCE

Field or microcosm studies may be used to directly show the occurrence of a specific natural attenuation process, as well as its ability to biodegrade contamination at a site (Wiedemeier et al., 1999). Microcosm studies are the only line of evidence that may provide definite mass balance when observing biodegradation of contaminants. Evidence from microcosm studies should be used at sites where proof of biodegradation of hydrocarbons cannot be supported by only soil and/or groundwater samples (Wiedemeier et al., 1995a).

Microcosm studies are undertaken under controlled conditions that do not mirror constant fluctuations experienced in the field. Measurements made in the laboratory may differ from field results. The causes for these discrepancies are complicated and often case specific, which requires further study. Thus, direct extrapolation of laboratory data to field conditions should be done with caution (Yeung et al., 1997). Ultimately the most important result obtained from microcosms is to document biological degradation as the primary natural attenuation process at a site (Wiedemeier et al., 1995a).

Several methods were used to examine microbial evidence at the study site. This included enumerating the indigenous microorganisms in the groundwater at the study site, and measuring microbial activity using microcosms under aerobic and anaerobic conditions. All methods used in this study are outlined below.

2.4.3.1 Microbial Enumeration

Prior to setting up an extensive microcosm study, the presence of biological activity in the groundwater was evaluated using the Biological Activity Reaction Test (BART™) (Droycon Bioconcepts Inc., 2000). This test was used to detect the activity and types of microorganisms in the subsurface.

Population densities of various microorganisms in groundwater were also estimated by the most probable number (MPN) technique. Growth or positive tests on a specific medium are recorded throughout the incubation time and statistical analysis is utilized to determine the extent of bacterial growth in the subsurface (Alef and Nannipieri, 1995).

2.4.3.2 Anaerobic Microcosms

Microcosms were used to assess the potential biodegradation rate of diesel under anaerobic conditions. Previous analysis at the study site found anaerobic conditions were dominant. Experiments were set-up to mirror the *in-situ* environment using laboratory microcosms. These experiments examined mineralization of the weathered free-product at the site under unamended in-situ conditions as well as with electron acceptor and nutrient amendment to enhance the process. Under all experimental conditions, mineralization of contaminant hydrocarbons were measured by the evolution of $^{14}CO_2$ from ^{14}C-labelled substrates (dodecane, toluene or acetate).

The effect of temperature on the anaerobic biodegradation rate of diesel was also explored. Degradation rates at the study site may be lower than in warmer climates as the groundwater temperature averages 8°C. Thus, one set of microcosms was incubated at room temperature (23°C) and the remainder were kept at a colder temperature of 10°C.

2.4.3.3 Geochemical and Contaminant Analysis

In addition to radiorespirometric measurements, anaerobic mineralization was measured by monitoring increases in methane (a by-product of methanogenesis) and decreases in electron acceptors (nitrate and sulfate). Methane evolution was detected on a gas chromatograph (detector and column type described in Section 5.6.2). Sets of microcosms were prepared to examine the use of electron acceptors under anaerobic conditions by microorganisms. Depletion of the electron acceptors nitrate and sulfate were monitored over time.

Large-scale microcosms (500 mL) were prepared to measure loss of contaminant concentration. Contaminant depletion was measured by monitoring decreases in contaminant substrates over time. Decreases in TEH concentrations were closely observed because these organics comprise a large proportion of diesel fuel.

Analysis of microbial metabolites was performed to confirm active degradation under anaerobic conditions in the groundwater (Gieg and Suflita, 2001). The presence of specific metabolites may indicate whether petroleum hydrocarbon contaminants are being biodegraded in the anaerobic subsurface environment.

2.4.3.4 Aerobic Microcosms

Aerobic biometer microbial activity experiments were performed to determine degradation rates in the presence of oxygen. Mineralization of radiolabelled ^{14}C-dodecane was observed by measuring production of $^{14}CO_2$ over time. The effects of nutrient amendment and temperature were also examined. Both unamended and nutrient-amended samples were incubated at either room temperature (28°C) or a colder temperature (10°C).

Aerobic mineralization experiments were important because they were more likely to produce rapid results, whereas anaerobic incubations have potentially long lag times before biodegradation is detected.

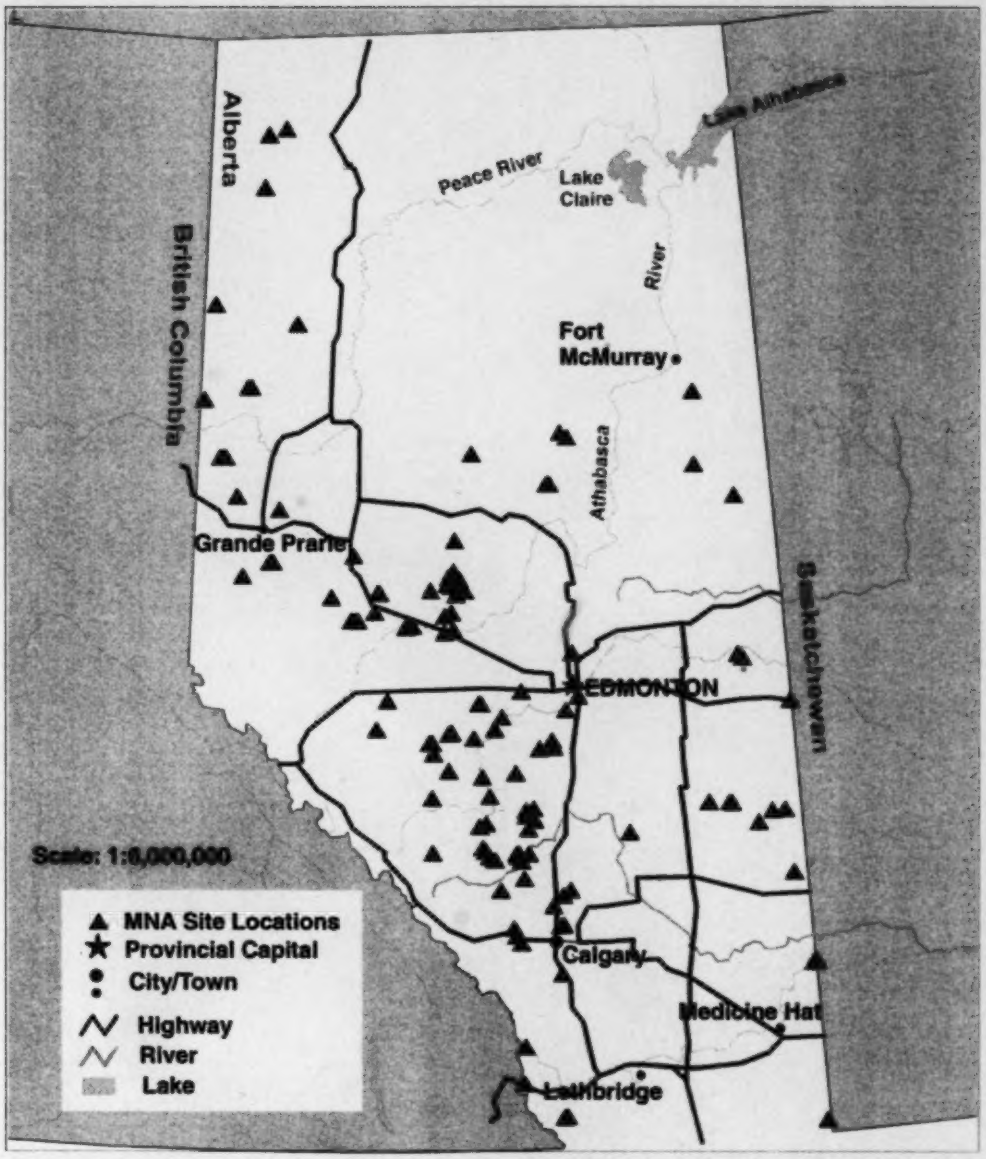

Alberta
British Columbia
Peace River
Lake Claire
Lake Athabasca
River
Fort McMurray
Athabasca
Grande Prarie
EDMONTON
Saskatchewan
Scale: 1:6,000,000
▲ MNA Site Locations
★ Provincial Capital
● City/Town
Highway
River
Lake
Calgary
Medicine Hat
Lethbridge

Figure 2-1. Geographical Distribution of Sites Investigated during Plume-a-thon Study

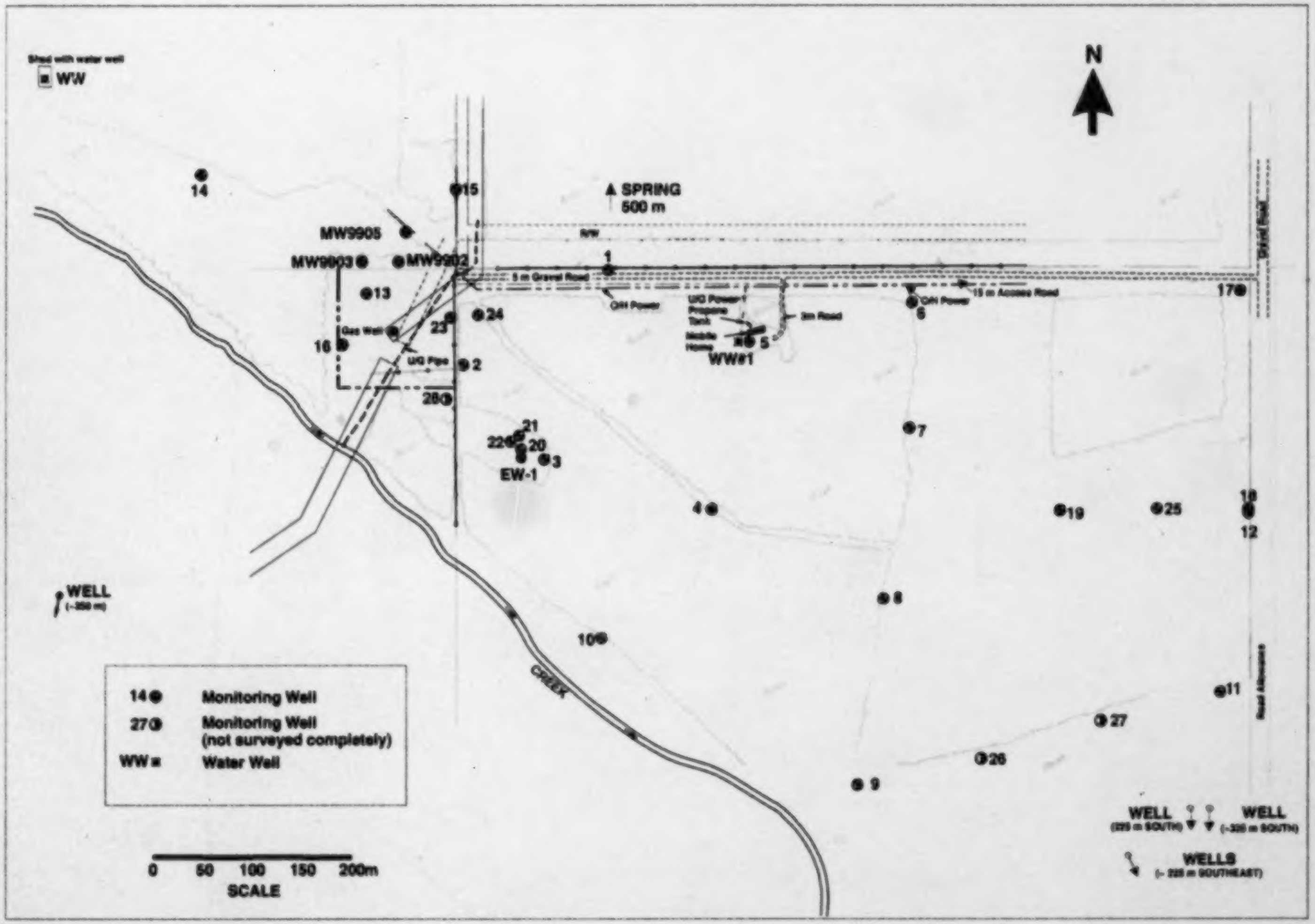

Shed with water well
WW
N
SPRING
500 m
14
15
MW9905
MW9903
MW9902
13
16
Gas Well
UG Pipe
23
24
2
28
R/W
1
5 m Gravel Road
OH Power
UG Power
Propane
Tank
Mobile
Home
5
WW#1
3m Road
6
OH Power
15 m Access Road
17
7
21
22
20
3
EW-1
4
19
25
18
12
8
10
CREEK
11
27
26
9
Road Allowance
WELL
(~350 m)
14 Monitoring Well
27 Monitoring Well (not surveyed completely)
WW Water Well
0
50
100
150
200m
SCALE
WELL
(225 m SOUTH)
WELL
(~320 m SOUTH)
WELLS
(~ 225 m SOUTHEAST)

Figure 2-2. Map of Study Site

3. LITERATURE REVIEW

All natural attenuation processes contribute to the reduction of PHC contaminant mobility. The abiotic and chemical transformation processes are described in Section 3.1.1. Biological processes are outlined in Section 3.1.2. Biodegradation or intrinsic bioremediation is the only process that will convert PHC contaminants to innocuous end-products. Chemical transformations only degrade halogenated hydrocarbons. Sections 3.2 to 3.6 focus on the microbial processes of intrinsic bioremediation and the effects of nutrients, temperature, LNAPL, free-phase fuel and co-contaminants. Section 3.7 discusses contamination and natural attenuation at upstream sites. There is emphasis on the components and effects of diesel contamination in Section 3.7.3 as this is the petroleum fuel of concern at the study site. Section 3.8 examines previous plume-a-thons that investigated trends at PHC contaminated sites.

3.1 NATURAL ATTENUATION (NA)

The mechanisms driving NA are divided into physical and biological processes. Physical processes do not destroy the contaminant mass but can cause a reduction in contaminant concentrations. An exception is chemical transformation, which can partially or completely degrade halogenated hydrocarbons. However, the degradation rates are typically slower than biological processes (Wiedemeier et al., 1999). Biological processes are the only natural attenuation mechanism that significantly destroys contaminant mass and retards the migration of the plume.

3.1.1 PHYSICAL PROCESSES

The fate and transport of a contaminant in the environment is controlled by the nature of an aquifer and the physical and chemical properties of each compound (Wiedemeier et al., 1995a). Evaluation of these processes at a site is crucial because they influence the distribution and concentrations of contaminants (Downey et al., 1999).

Many abiotic processes including advection, dispersion, sorption, dilution, volatilization and chemical transformations affect the fate and transport of PHC compounds dissolved in the groundwater; these processes are described below.

3.1.1.1 Advection

Advective transport refers to the movement of solute by bulk groundwater movement. Dissolved contaminant migration in the subsurface is driven mainly by advection. Advective transport can be illustrated by considering a solute that does not react chemically or biologically in the subsurface and moves at the same velocity as the groundwater. The rate at which the solute moves through the subsurface is dependent on aquifer properties including hydraulic conductivity, effective porosity, and the hydraulic gradient (National Research Council, 2000). However, contaminant properties do not affect the advective process (Wiedemeier et al., 1995a).

3.1.1.2 Dispersion

Hydrodynamic dispersion in groundwater is the process where a contaminant plume spreads out in all directions from the primary direction of plume migration. Dispersion of dissolved organic compounds in the subsurface results in reduced contaminant concentrations and introduces contaminants into previously pristine areas of an aquifer (Wiedemeier et al., 1999).

There are two components of hydrodynamic dispersion: mechanical dispersion and molecular diffusion (Downey et al., 1999). Mechanical dispersion is mixing that occurs as a result of local variations in velocity and tortuous flow paths. Variations of rate and direction of transport velocities are due to aquifer characteristics, such as hydraulic conductivity and porosity of the surrounding soils (Wiedemeier et al., 1999).

Mechanical dispersion is the dominant process that causes hydrodynamic dispersion. Molecular diffusion is usually insignificant except when groundwater velocities are extremely slow (Wiedemeier et al., 1999). Molecular diffusion occurs when solutes migrate from an area of high concentration to an area of low concentration. Diffusion is dependent on contaminant properties, the soil properties and the concentration gradient in the aquifer (Wiedemeier et al., 1995a).

3.1.1.3 Sorption

Sorption involves dissolved contaminants adhering to soil and sediment particles that comprise the aquifer matrix (US EPA, 1999). Sorption can slow or stop the movement of contaminants, because the aquifer materials temporarily prevent contaminant mass moving further downstream (National Research Council, 2000).

The degree of sorption is dependent on the composition of the aquifer matrix including organic carbon content, clay mineral content, bulk density, specific surface area, and total porosity. Contaminant properties are also important, specifically solubility and the octanol-water partitioning coefficient (K_{ow}) (Wiedemeier et al., 1995a).

3.1.1.4 Dilution

Dilution or recharge takes place when infiltration water enters the groundwater system via discharge from surface water bodies or infiltration from precipitation. Additional water entering an area where there is a dissolved contaminated subsurface plume will dilute the contaminants and may provide an influx of electron acceptor charged water. Recharge of electron acceptors will affect the geochemical processes and increase the rate of natural attenuation (Wiedemeier et al., 1999).

3.1.1.5 Volatilization

Volatilization is not a destructive mechanism, but does remove contaminant mass from soil and groundwater. Volatilization of a contaminant into the gas phase depends on the contaminant vapour pressure, solubility and Henry's law constant (Wiedemeier et al., 1999; Downey et al., 1999). Many petroleum hydrocarbons readily evaporate into the atmosphere, which can reduce the concentration in groundwater. Once hydrocarbons are in the atmosphere, they can be broken down by sunlight (US EPA, 1999). BTEX has the highest volatility of aromatic hydrocarbons. The high solubility and volatility of BTEX accounts for the mobility of these compounds and their ability to readily dissolve in groundwater (US EPA, 1999).

3.1.1.6 Chemical Transformations

Chemical transformations degrade contaminants without requiring the presence of microorganisms. However, only halogenated compounds undergo these reactions in groundwater. Two types of common chemical transformation reactions are hydrolysis and dehalogenation. Hydrolysis is a substitution reaction in which a compound such as 1,1,1-Trichloroethane (1,1,1-TCA) reacts with water and a halogen substituent is replaced with a hydroxyl (OH^-) group (Wiedemeier et al., 1999). The reaction products include alcohols and alkenes that may be more susceptible to biodegradation (Wiedemeier et al., 1999).

Dehydrodehalogenation is a two-step elimination reaction that involves the removal of a halogen and a hydrogen atom from carbon atoms on halogenated alkanes. The product of this two-step reaction is an alkene. Contrary to hydrolysis, the likelihood of dehydrodehalogenation reactions increases with the number of halogen substituents (Wiedemeier et al., 1999).

Hydrogenolysis and dihaloelimination reactions are two abiotic reductive dechlorination reactions that may occur in groundwater. Hydrogenolysis involves the replacement of a halogen (*e.g.* chlorine) with hydrogen, whereas dihaloelimination removes two halogens and forms a carbon-carbon double bond. It is not clear whether these reactions are truly abiotic because of their reliance on microbial activity to produce reducing conditions or reactions. Thus, these reactions may be classified as a form of cometabolism (Wiedemeier et al., 1999).

3.1.2 BIOLOGICAL PROCESSES

Many components of PHCs such as BTEX and some PAHs (polynuclear aromatic hydrocarbons) can be biodegraded by microorganisms indigenous to the subsurface (US EPA, 1999). Many microorganisms derive energy for cell growth and reproduction via respiration, which involves oxidation-reduction reactions with inorganic TEAs. Hydrocarbons such as BTEX are used as a substrate (food source) and are oxidized as an electron donor, while one or more of a series of electron acceptors are reduced. Oxygen is readily used as an electron acceptor during aerobic respiration because it provides the highest energy yield. Once oxygen is depleted, other TEAs are used in the following order of preference: nitrate, manganese (IV), iron (III), sulfate and carbon dioxide (Downey et al., 1999). The amount of energy obtained from oxidation of a contaminant is proportional to the reduction potential of the electron acceptor (Nies and Kapoor, 1999). This means that each successive electron acceptor provides less energy for the subsurface microorganisms and the redox potential in the groundwater decreases.

Biodegradation results in measurable changes in the groundwater chemistry. Numerous authors have documented the corresponding geochemical changes during the intrinsic bioremediation of PHCs (Borden, 1994; Suthersan, 1997; Wiedemeier et al., 1999; Chapelle, 2001), for example, production of H_2S from sulfate reduction, or of reduced forms of manganese and iron.

3.2 MICROBIOLGICAL DEGRADATION PROCESSES

Over the past 20 years, laboratory and field studies have shown that indigenous microorganisms with the ability to degrade a wide variety of hydrocarbons are ubiquitous in the subsurface (Wiedemeier et al., 1999). These microorganisms are well adapted to the physical and chemical conditions of this environment (Borden et al., 1995; Kennedy et al., 2001). Many microorganisms degrade organic substrates when sufficient electron acceptors and nutrients are available and use electron transport systems and chemiosmosis to generate energy in the form of adenosine triphosphate (ATP) and oxidize organics to CO_2. (Chapelle, 2001). Some anaerobes, such as fermenters, produce a variety of end products including acetate, lactate, formate, CO_2 and alcohols (Chapelle, 2001).

The amount of energy released from a redox reaction or energy required to drive a reaction to completion is quantified as Gibbs free energy of reaction (ΔG_r). Microorganisms couple the oxidation of an electron donor with the reduction of an electron acceptor to produce an overall reaction that is energy yielding. Table 3-1 summarizes the overall ΔG_r for aerobic respiration, denitrification, manganese (IV) reduction, iron (III) reduction, sulfate reduction and methanogenesis. It is thermodynamically favourable to use electron acceptors that provide the greatest amount of free energy during respiration. Oxidation-reduction reactions will generally occur in order of their thermodynamic energy yield as long as there are adequate electron acceptors and microorganisms to facilitate each reaction (Wiedemeier et al., 1999).

Table 3-1. Gibbs Free Energy of the Reaction (ΔG_r) for Microbial Mediated Redox Reactions (Wiedemeier et al., 1995a; Downey et al., 1999).

Terminal Electron Accepting Process	ΔG_r kJ/mole Benzene	ΔG_r kJ/mole Toluene	ΔG_r kJ/mole Ethylbenzene	ΔG_r kJ/mole m-Xylene
Aerobic Respiration	-3202	-3823	-4461	-4448
Denitrification	-3245	-3875	-4522	-4509
Manganese (IV) Reduction	-3202	-3824	-4461	-4449
Iron (III) Reduction	-2343	-2792	-3257	-3245
Sulfate Reduction	-414.3	-597.7	-697.7	-685.6
Methanogenesis	-135.6	-142.6	-166.7	-154.6

As each successive electron acceptor is depleted, the groundwater becomes more reducing and the redox potential decreases. The redox potential (Eh) is a measure of electron activity and indicates the relative tendency of a solution to accept or transfer electrons (Wiedemeier et al., 1999). Table 3-2 summarizes the redox potentials for various electron acceptors. An overall reduction in the redox potential of groundwater provides less energy to the system and allows successive electron acceptors to be facilitated by microorganisms adapted to specific redox capacities. For example, once oxygen and nitrate are depleted in an aquifer, the redox potential is reduced to a level at which iron (II) reduction can occur (Wiedemeier et al., 1995a). Furthermore, microorganisms can only oxidize hydrocarbons such as BTEX using electron acceptors that have a higher redox potential than BTEX (-300mV). Aerobic respiration, denitrification, manganese (IV) reduction, iron (III) reduction, sulfate reduction and methanogenesis all have higher redox potentials than each of the BTEX components (Wiedemeier et al., 1995a).

Table 3-2. Electron Acceptor Half-Cell Reactions and Redox Potentials at pH = 7 and T = 25°C (Wiedemeier et al., 1995a)

Electron Acceptor	Reaction	Redox Potential (Eh^{o})*
Oxygen	$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$	+820 mV
Nitrate	$2NO_3^- + 12H^+ + 10e^- \longrightarrow N_2 + 6H_2O$	+740 mV
Manganese (IV)	$MnO_{2(s)} + HCO_3^- + 3H^+ + 2e^- \longrightarrow MnCO_3 + 3H^+ + 2H_2$	+520 mV
Iron (III)	$FeOOH_{(s)} + HCO_3^- + 2H^+ + e^- \longrightarrow FeCO_{3(s)} + 2H_2O$	-50 mV
Sulfate	$SO_4^{2-} + 9H^+ + 8e^- \longrightarrow HS^- + 4H_2O$	-220 mV
Carbon Dioxide	$CO_2 + 8H^+ + 8e^- \longrightarrow CH_4 + 2H_2O$	-230 mV

For complete degradation of PHCs to occur, there must be a mixed microbial population containing microorganisms capable of adapting to the changing geochemistry (*i.e.* redox potential, electron acceptor availability) of the subsurface (Borden et al., 1995). The various microbial processes that biologically degrade hydrocarbon contamination are described below.

3.2.1 AEROBIC DEGRADATION

Biodegradation processes are generally classified as either aerobic or anaerobic, with aerobic utilizing oxygen as the TEA. Microaerobic biodegradation is another type of aerobic reaction that occurs when the environment is anaerobic but a local area in the groundwater is receiving a low concentration of oxygen (Armstrong et al., 2000).

The majority of aromatic and aliphatic hydrocarbons are biodegradable under aerobic conditions. Complete aerobic mineralization of many hydrocarbons to carbon dioxide and water initially requires the addition of molecular oxygen to the hydrocarbon. Oxygenase enzymes facilitate this initial stage of hydrocarbon metabolism (Nies and Kapoor, 1999). Dissolved oxygen (DO) is later used as an electron acceptor for energy generation. The availability of dissolved oxygen is controlled by aquifer reaeration, which depends on groundwater flow, rainfall events and capillary fringe aeration (Salanitro et al., 1997). The major limitation on aerobic biodegradation in the subsurface is the low solubility of oxygen in water (~10 mg/L). Once oxygen is depleted there are many alternatives for use as electron acceptors (Nies and Kapoor, 1999).

The following reaction shows the overall stochiometry of benzene degradation using DO, from which the amount of DO required to metabolize 1 mole of benzene can be calculated.

$$C_6H_6 + 7.5O_2 \rightarrow 6CO_2 + 3H_2O \qquad [1]$$

This reaction indicates that 7.5 moles of DO are required to degrade 1 mole of benzene. On a mass basis this is:

Mass benzene = 78g/mol x 1 mole = 78g

Mass Oxygen = 32 g/mol x 7.5 mole = 240g

The mass ratio of oxygen required to degrade 1 mole of benzene is:

240:78 = 3.08:1

Thus, 3.08 mg DO is required to completely metabolize 1 mg of benzene to CO_2 and H_2O in the absence of microbial cell production. This calculation can be made for toluene, ethylbenzene and total xylenes. The amount of oxygen used to degrade BTEX can be determined by averaging the oxygen consumed for each separate compound. Thus, biodegradation of 1 mg of total BTEX consumes approximately 3.14 mg of oxygen. Water saturated with air contains 6 to 12 mg/L of DO, which can biodegrade 2 to 4 mg/L of dissolved BTEX by strictly aerobic processes. If the hydrocarbon concentration exceeds this, then oxygen will be depleted and biodegradation must proceed by other TEAPs (Borden, 1994). Table 3-3 summarizes the mass of electron acceptor used or metabolic by-product produced during biodegradation of 1 mg of BTEX for each TEAP.

Table 3-3. Mass (mg) of Electron Acceptor That is Used or Metabolic By-Product Produced during Degradation of 1 mg of Total BTEX[1]

Terminal Electron Accepting Process	Mass of electron acceptor used (mg)	Mass of metabolic by-product produced (mg)
Aerobic respiration	3.14	
Denitrification	4.9	
Manganese reduction		10.5
Iron Reduction		21.8
Sulfate Reduction	4.7	
Methanogenesis		0.78

1 - Average of all BTEX compounds based on individual stochiometry

Table 3-3 assumes complete mineralization of BTEX, however, a certain fraction of contaminant may not be fully oxidized and produce metabolic intermediates instead. Furthermore, some organic substrate can be incorporated into biomass through anabolic processes with little or no oxidation (Kennedy et al., 2001). Thus, the stochiometric prediction of contaminant mass destroyed is a conservative estimate.

3.2.2 NITRATE REDUCTION

Electrical storms oxidize large amounts of N_2 to N_2O_5. This form of nitrogen reacts with water to form HNO_3, which falls to earth in raindrops. Another source of nitrate is microbial nitrification, where nitrite and nitrate are produced from ammonia. Nitrate is also produced by direct oxidation of nitrogen or ammonia in the manufacture of chemical fertilizers (Sawyer et al., 1994).

Once available DO has been consumed, facultative anaerobic microorganisms may use nitrate (NO_3^-) as a TEA in a process called denitrification. Many species of denitrifying microorganisms are capable of oxidizing organic substrates. The principal end products of microbial degradation via denitrification and nitrate reduction are carbon dioxide, water and nitrogen gas (N_2) (Chapelle, 2001). The denitrification of nitrate to N_2 proceeds in the following sequence:

$$NO_3^- \Longrightarrow NO_2^- \Longrightarrow NO \Longrightarrow N_2O \Longrightarrow N_2$$

Most denitrifiers have the ability to reduce NO_3^- through the entire pathway to N_2, but some strains lack the ability to reduce $NO_3^- \Longrightarrow NO_2^-$ and $N_2O \Longrightarrow N_2$, and others lack the ability to reduce nitrite (Tiedje, 1988).

Degradation of BTEX by denitrification is thermodynamically more favourable than aerobic respiration (Table 3-1). However, facultative aerobes that can use both oxygen and nitrate as electron acceptors will only use nitrate once the groundwater system has been depleted of dissolved oxygen (Wiedemeier et al., 1995a). The presence of oxygen inhibits synthesis of an enzyme (nitrate reductase) that transfers electrons to nitrate (Nies and Kapoor, 1999). Thus, denitrification is restricted to anaerobic or microaerobic environments (Chapelle, 2001).

PHC plumes generally do not contain nitrate, which indicates this electron acceptor is rapidly depleted once the groundwater becomes anaerobic. This suggests that availability of nitrate as opposed to the microbial process is a limiting factor during biodegradation (Wiedemeier et al., 1999). Thus, active denitrification requires the presence of sufficient concentrations of nitrate, denitrifying bacteria, biodegradable organic carbon, and slightly reducing conditions (Wiedemeier et al., 1999).

Complete degradation of benzene via denitrification is represented stochiometrically by:

$$6NO_3^- + 6H^+ + C_6H_6 \rightarrow 6CO_2 + 6H_2O + 3N_2 \qquad [2]$$

The mass ratio is calculated in the same manner as oxygen. For every milligram of benzene metabolized, 4.77 mg of nitrate is reduced. Table 3-3 shows the average ratio of nitrate reduced per milligram of BTEX is 4.9:1.

Anaerobic biodegradation under denitrifying conditions in laboratory studies has been well documented (Kuhn et al., 1988; Evans et al., 1991b; Ball and Reinhard, 1996). There are also a number of studies that demonstrate the effectiveness of denitrification under *in-situ* conditions (Major et al., 1988; Hutchins et al., 1991; Starr and Gillham, 1993).

3.2.3 IRON AND MANGANESE REDUCTION

Iron (III) and manganese (IV) reduction are important mechanisms for oxidizing natural or contaminant organic compounds in diverse aquatic environments (Lovley, 1991). After available oxygen and nitrate are depleted in the groundwater, some microorganisms can oxidize organic contaminants using insoluble Fe (III) as an electron acceptor (Borden et al., 1995). Iron is the fourth most abundant element in the earth's crust, thus large amounts may be present in the sediments of most aquifers to act as potential electron acceptors (Borden et al., 1995; Kennedy et al., 2001). Fe (III) in the form of amorphous and poorly crystalline Fe (III) hydroxides, Fe (III) oxyhydroxides, and Fe (III) oxides, are best for microbiological reduction (Lovley, 1991; Borden et al., 1995).

Oxidation of benzene by Fe (III) reduction is stochiometrically represented as:

$$60H^{+} + 30Fe(OH)_3 + C_6H_6 \rightarrow 6CO_2 + 30Fe^{2+} + 78H_2O \qquad [3]$$

Mass ratio calculations conclude that 41.1 mg of $Fe(OH)_3$ are required to completely metabolize 1 mg of benzene. The end-product, Fe (II), is the parameter measured in groundwater monitoring to assess geochemical change in the subsurface. Fe (II) is more water-soluble than Fe (III), and thus can be readily measured to determine the extent of Fe (III) reduction. Mineralization of 1 mg of benzene by Fe (III) reduction produces 21.5 mg of Fe (II). Table 3-3 gives the amount of Fe (II) produced for 1 mg of oxidized BTEX (21.8 mg) (Wiedemeier et al., 1995a).

High concentrations of Fe (II) in groundwater were originally attributed to nonenzymatic reduction of Fe (III) oxyhydroxides, which are thermodynamically unstable in anaerobic environments where organic compounds are present. However studies indicate active Fe (III) reduction requires Fe (III)-reducing bacteria in the groundwater that are in direct contact with iron, biologically available Fe (III), sufficient biodegradable organic carbon, and reducing conditions (Lovley, 1991; Wiedemeier et al., 1999).

Fe (III) reduction is inhibited by the presence of nitrate. This may be due to lower hydrogen concentrations when nitrate is available compared to when Fe (III) reduction is the predominant TEAP. Also, nitrate-reducing microorganisms may outcompete Fe (III)-reducers (Lovley, 1991). A lack of Fe (II) accumulation can sometimes occur in the presence of Mn (IV), indicating possible Fe (III) reduction inhibition. However, Lovley and Philips (1988) reported Fe (III)-reducing microorganisms appeared to reduce Fe (III) in the presence of Mn (IV). The lack of Fe (II) accumulation is attributed to nonenzymatic oxidation of Mn (IV) by Fe (II) (Lovley and Philips, 1988). In addition Fe (III) may not be preferentially used because there is a lower concentration of electron donors under Mn (IV) reducing conditions (Lovley, 1991).

Unlike Fe (III), the presence of nitrate does not inhibit Mn (IV). Mn (IV) reduction provides more energy to microorganisms than Fe (III) reduction (Table 3-1) and has a higher redox potential. Also, Mn (IV) is more reactive with organic compounds than Fe (III) (Lovley, 1991). This means that Mn (IV) reduction is likely to occur prior to Fe (III) reduction in the groundwater.

Mineralization of benzene via Mn (IV) reduction is represented stochiometrically as follows:

$$C_6H_6 + 30H^+ + 15MnO_2 \rightarrow 6CO_2 + 18H_2O + 15Mn^{2+} \qquad \textbf{[4]}$$

Degradation of 1 mg of benzene under manganese reducing conditions will reduce 16.7 mg of MnO_2 (Mn (IV). As with Fe (III) reduction, the end-product Mn (II) is the parameter measured in groundwater because it is more water-soluble than Mn (IV).

Oxidation of 1 mg of benzene by Mn (IV) reduction will produce 10.6 mg of Mn (II). The mass of metabolic by-product to biodegrade 1 mg of BTEX by Mn(IV) reduction is given in Table 3-3.

The presence of Fe (III) and/or Mn (IV) appears to inhibit active sulfate reduction and methane production. This indicates that microorganisms which reduce Fe (III) and Mn (IV) outcompete sulfate reducers and methanogens for electron donors in the subsurface (Lovley, 1991). Furthermore key intermediates of anaerobic decay, such as hydrogen and acetate, are kept at concentrations that are too low for the competing processes (Suflita et al., 1997).

3.2.4 SULFATE REDUCTION

Sulfate-reducing bacteria are ubiquitously distributed in the environment. As a group they can tolerate a wide variety of environmental conditions including temperatures ranging from 0 to 110 °C and pH values from 3 to 9, extremes of salinity, redox status and degrees of oxygenation. In addition to sulfate, sulfate-reducers (depending on the particular organism) can use a variety of different electron acceptors such as thiosulfite, sulfite, tetrathionate, dithionate, elemental sulfur, nitrate, halo- or nitroaromatic compounds, malate, or fumarate (Suflita et al., 1997). It has been discovered that many sulfate reducers are tolerant of low levels of molecular oxygen, however high O_2 is still believed to be deleterious to their survival.

Once all available oxygen and nitrate in the groundwater have been depleted, sulfate-reducing bacteria can facilitate biodegradation of PHCs (Wiedemeier et al., 1999). Sulfate reduction occurs when sulfate-reducing bacteria are present in the contaminated aquifer, sulfate is present, there is sufficient biodegradable carbon and reducing conditions prevail (Wiedemeier et al., 1999). Sulfate reduction continues until usable carbon or sulfate is depleted, limiting bacterial activity (Azadpour-Keeley et al., 1999).

Benzene metabolism by sulfate reduction is represented stochiometrically by:

$$7.5H^{+} + 3.75SO_4^{2-} + C_6H_6 \rightarrow 6CO_2 + 3.75H_2S + 3H_2O \qquad [5]$$

Using the equation, 4.6 mg of sulfate is required to completely metabolize 1 mg of benzene (Wiedemeier et al., 1995a). The amount of sulfate required to degrade 1 mg of BTEX is given in Table 3-3.

The reduced product of sulfate-reduction, HS^-, is often precipitated as an iron sulfide, which is evidenced by blackening of surrounding sediments (Kennedy et al., 1999). This precipitate and the characteristic odour of hydrogen sulfide gas indicate rapid sulfate reduction in an anaerobic environment (Suflita et al., 1997).

Sulfate reduction of PHCs has been actively observed in a number of field (Davis et al., 1999; Anderson and Lovely, 2000; Cunningham et al., 2001) and laboratory studies (Edwards et al., 1992; Beller et al., 1992; Lovley et al., 1995; Ball and Reinhard, 1996; Chapelle et al., 1996). Numerous microbiological studies have characterized sulfate reducers that mineralized PHCs and examined the metabolism of environmental contaminants by sulfate reducing bacteria (Aeckersberg et al., 1991; Rueter et al., 1994; Ensley and Suflita, 1999; So and Young, 1999b; Meckenstock et al., 2000; Wilkes et al., 2000; Kropp et al., 2000).

3.2.5 METHANOGENESIS

Once the more energetically favourable electron acceptors are depleted or unavailable in the groundwater, carbon dioxide can be used as a terminal electron acceptor and reduced via methanogenesis to form acetate or methane (Suflita et al., 1997). Methanogenesis can occur through two distinct pathways involving a combination of fermentative and respiratory microorganisms in the anaerobic subsurface environment.

The first pathway is a two-step process involving fermentation and respiration. First, organic compounds are fermented to acetate, hydrogen, carbon monoxide and formate (Wiedemeier et al., 1999; Chapelle, 2001). The anaerobes that are involved in this step are fermentative microorganisms not methanogens. The stochiometric representation of fermentation of benzene to acetate is represented as:

$$C_6H_6 + 6H_2O \rightarrow 3CH_3COOH + 3H_2 \qquad [6]$$

The products of fermentation still contain usable energy that can be tapped by respiratory microorganisms (*i.e.* methanogens) (Chapelle, 2001). The second step of this methanogenic pathway involves the reduction of acetate, which is represented stochiometrically as:

$$3CH_3COOH \rightarrow 3CH_4 + 3CO_2 \qquad [7]$$

The second methanogenic pathway uses CO_2 as an electron acceptor and the H_2 produced during fermentation (Equation 6) as an electron donor. Methanogenic microorganisms accomplish this reaction over four steps where hydrogen is sequentially reacted with carbon. This process yields slightly more energy than using acetate as an electron acceptor (Chapelle, 2001). This is represented stochiometrically by:

$$3H_2 + 0.75CO_2 \rightarrow 0.75CH_4 + 1.5H_2O \qquad [8]$$

The overall stochiometric equation taking both methanogenic pathways into consideration is:

$$C_6H_6 + 4.5H_2O \rightarrow 2.25CO_2 + 3.75CH_4 \qquad [9]$$

Therefore, 1 mg of biodegraded benzene produces 0.77 mg of methane and 1 mg of BTEX produces 0.78 mg of methane (Wiedemeier et al., 1995a).

Although methanogenesis provides minimal energy to microorganisms the TEAP will proceed when methanogenic bacteria are present, carbon dioxide is available, there is sufficient suitable organic carbon (*i.e.* acetate, CO_2 and formate), and strongly reducing conditions are prevalent (Wiedemeier et al., 1999). Degradation of PHCs via methanogenesis has been demonstrated in a number of laboratory studies. Toluene and benzene degradation, and toluene and *o*-xylene mineralization were observed under methanogenic conditions by Grbic-Galic and Vogel (1987), and Edwards and Grbic-Galic (1994), respectively. Wilson et al. (1986) also examined active degradation of benzene, toluene and xylene in a methanogenic aquifer.

Addition of more thermodynamically favourable electron acceptors, such as nitrate and sulfate, does not necessarily increase PHC degradation in contaminated aquifers under methanogenic conditions. This is likely because the indigenous microbial populations are acclimated to the conditions of methanogenic fermentation (Edwards and Grbic-Galic, 1994).

3.2.6 HYDROGEN

When aquifers become contaminated with PHCs, it can be difficult to determine the distribution of TEAPs. Redox reactions in contaminated groundwater are commonly evaluated in terms of equilibrium thermodynamics and redox potential. However, these measures are not reliable in practice because redox equilibrium is rarely achieved in low temperature environments (Lovley et al., 1994).

Another approach that can be used to assess the dominant TEAP in contaminated areas is measuring hydrogen gas concentrations. Hydrogen is a dynamic molecule in anaerobic environments and measuring hydrogen gas concentrations in groundwater can indicate the dominant TEAP (Gieg et al., 1999b). Hydrogen is an important intermediate in microbial redox reactions. Furthermore, when TEAPs are divided into distinct zones there is a definitive relationship between required hydrogen concentrations and the predominant TEAPs in the subsurface (Lovley et al., 1994). Hydrogen concentrations required for the various electron acceptors are shown in Table 3-4.

Table 3-4. Hydrogen Concentrations Required for Terminal Electron Accepting Processes (Lovley et al., 1994; Wiedemeier et al., 1999; Chapelle, 2001)

Terminal Electron Accepting Process	Hydrogen Concentration (nanomoles per litre)
Aerobic Respiration	<0.1
Denitrification	0.1
Mn (IV) Reduction	0.1
Iron (III) Reduction	0.2 – 0.8
Sulfate Reduction	1 – 4
Methanogenesis	5 – 20

3.2.7 EXPRESSED BIODEGRADATION CAPACITY

Evaluation of TEAPs is critical to determining whether natural attenuation is feasible at a site. Stochiometric relationships of TEAPs are used in conjunction with changes in geochemical indicators in the groundwater to estimate the relative importance of biodegradation processes and the biodegradation capacity of electron acceptors in the groundwater. The biodegradation capacity at a site is the amount of contamination that can be completely degraded based on the electron-accepting capacity of groundwater. Each TEAP has the capacity to degrade a certain amount of contaminant depending on the electrons transferred during respiration. This is known as the expressed biodegradation capacity (EBC) (Wiedemeier et al., 1999).

Groundwater at a site is continually receiving additional electron acceptors from the upstream groundwater entering the plume and from infiltration from the ground surface. This means that biodegradation capacity is not a fixed value but it can fluctuate depending on the groundwater flow. Therefore, EBC is used as a semi-quantitative method to determine the potential for biodegradation (Wiedemeier et al., 1999).

The mass ratios described in the previous section are summarized in Table 3-3. These mass ratios are used as BTEX utilization factors when calculating the amount of BTEX biodegraded by a given TEAP (Wiedemeier et al., 1999). The absolute differences in indicator concentrations between background and plume groundwater are used to estimate the theoretical concentration of BTEX that can be degraded by each TEAP. This is expressed by the following equation:

$$EBC_x = \frac{\left|C_B - C_P\right|}{F} \qquad [10]$$

where, EBC_x = expressed biodegradation capacity for given terminal electron accepting process [mg/L]

C_B = average background electron acceptor or metabolic by-product concentration [mg/L]

C_P = Concentration [mg/L] of electron acceptor or metabolic by-product within the plume

F = BTEX utilization factors: The mass [mg] of electron acceptor or metabolic by-product that is used or produced during degradation of 1 mg of total BTEX (Table 3-3).

The following is a sample calculation to illustrate how to determine the amount of BTEX biodegraded by a terminal electron accepting process:

$$EBC_{sulfate} = \frac{\left|C_{B(sulfate)} - C_{P(sulfate)}\right|}{F} \qquad [11]$$

where, EBC_x = expressed biodegradation capacity for sulfate reduction [mg/L]

C_B = average background sulfate concentration [mg/L] = 100mg/L

C_P = Concentration [mg/L] of sulfate within the plume =10 mg/L

F = The mass [mg] of sulfate used during degradation of 1 mg of total BTEX = 4.7

$$EBC_{sulfate} = \frac{100 - 10}{4.7} = 19.1 \text{ mg/L}$$

Thus, if the contaminant plume has 10 mg/L of sulfate compared to a background level of 100 mg/L, then sulfate reduction could theoretically have assimilated 19.1 mg/L of total BTEX.

Interpretations of EBC should be considered semi-quantitative because the production of cell mass is not taken into account. Furthermore, complete mineralization of contaminants is assumed and initial degradation of compounds into intermediates is not captured (Komex International Ltd., 2001b).

3.3 METABOLIC INTERMEDIATES

Microbial metabolites can be used as reliable indicators of active biodegradation in the subsurface. Studies have identified unique intermediate metabolites of PHC degradation and the pathways being utilized to reach complete mineralization. These can be used as an aid in determining whether the dominant TEAP is effectively degrading contaminants.

Aerobic microorganisms initiate biodegradation of many compounds by inserting molecular oxygen into a hydrocarbon using oxygenase enzymes to form mono- and dihydroxylated compounds (Nies and Kapoor, 1999). Monooxygenase enzymes are used in the metabolic pathways of alkanes, whereas monooxygenases and dioxygenases initially attack aromatic hydrocarbons (Heider et al., 1999). These oxygenases incorporate oxygen ($\frac{1}{2}$ O_2) into alkanes to produce alcohols, which are subsequently oxidized to corresponding fatty acids (Figure 3-1). Aromatics incorporate oxygen to form phenolic compounds, which are precursors for oxidative ring cleavage (Heider et al., 1999). Further oxidation proceeds through *ortho* and *meta* cleavage pathways, which are illustrated in Figure 3-1. The metabolic pathways of alkanes and aromatics produce organic acids that can be further degraded to carbon dioxide via beta oxidation and the tricarboxylic acid (TCA) cycle (Figure 3-2) (Chapelle, 2001).

Anaerobic bacterial metabolism of toluene, xylenes and ethylbenzene is initiated by the addition of their alkyl group to the double bond of fumarate, which forms an activated molecule containing succinic acid (Gieg and Suflita, 2001; Elshahed et al., 2001). For example, the addition of toluene to fumarate forms benzylsuccinic acid, which is identified as a transient intermediate of anaerobic toluene oxidation (Heider et al., 1999). Benzylsuccinic acid is further oxidized to benzoic acid (or its CoA-thioester) which is recognized as a central intermediate in anaerobic degradation of many aromatic compounds (Heider et al., 1999). Proposed pathways for the degradation of toluene, xylenes and ethylbenzene under anaerobic conditions are presented in Figure 3-3 (Elshahed et al., 2001). In addition, further oxidation of benzoic acid and toluic acid are also illustrated.

Kropp et al. (2000) observed dodecylsuccinic acid metabolites during anaerobic oxidation of *n*-dodecane. This suggests that the bond between dodecane and succinate was formed by an addition reaction across the double bond of fumarate. This reaction is analogous to the aromatic methyl group addition to fumarate. Kropp et al. (2000) hypothesized that dodecylsuccinic acid metabolites may be further metabolized to 2-carboxy-substituted cellular fatty acids. Long chain fatty acids converted from *n*-alkanes are fed into an oxidation pathway known as the beta-oxidation pathway to ultimately produce ATP and CO_2 (Chapelle, 2001).

The metabolic intermediates described above can be used to support evidence of biodegradation in the subsurface. Some metabolites such as toluic acid and phthalic acid may be intermediates of aerobic or anaerobic xylene or toluic acid degradation, respectively (Elshahed et al., 2001). However, detection of these intermediates in anaerobic microcosms and groundwater can be used with other evidence to confirm anaerobic degradation (Elshahed et al., 2001).

3.4 EFFECTS OF NUTRIENTS

Nutrients are often a limiting factor in contaminated plumes, especially when contamination produces a large amount of carbon relative to available nitrogen (N) and/or phosphorus (P) (Braddock et al., 2001). The addition of nutrients such as nitrogen and phosphorous to nutrient-limited oil degrading bacteria in the subsurface can help stimulate biodegradation. Phosphorous is required by microorganisms for the biosynthesis of nucleic acids (DNA and RNA), ATP and other cellular components (Mills and Frankenberger Jr, 1994). Nitrogen is also used in nucleic acids, and in amino acids which are building blocks for proteins.

Nutrient limitation may be due to either low nitrogen or phosphorous concentrations or a combination of both. Braddock et al. (1997) observed the relationship between N and P and found that nitrogen appeared to be the major limiting nutrient in contaminated surface soil. Although N greatly stimulated the biodegradation of hydrocarbons, the addition of P to N-supplemented soils resulted in further enhancement of hydrocarbon degradation (Braddock et al., 1997). This is the result of a synergistic relationship between nitrogen and phosphorous. For example, increased application of nitrogen fertilizer will increase phosphorous uptake by plants growing in low or high phosphorous soil, and application of phosphorous increases nitrogen uptake (Walworth and Reynolds, 1995).

Aquifers with a large organic N component may require inorganic N supplements to stimulate biodegradation. This is because organic N may not be functionally bioavailable over the time frame required for bioremediation (Graham et al., 1999). External inorganic nutrient addition can aid in providing adequate nutrients to stimulate biodegradation of hydrocarbon contaminants (Graham et al., 1999). However, Graham et al. (1999) found that oversupplying one nutrient relative to the other did not increase the rate of biodegradation. Thus, nutrients were potentially wasted.

In some cases nutrients may have an inhibitory effect on degradation. Walworth et al. (1997) observed that dry soil was easily overfertilized, whereas wet soil was less sensitive to excessive N application. In dry soil, soluble N salts are partitioned into a smaller volume of water. Wetter soil can effectively dilute N because there is a large volume of water, which decreases the effect of soil solution N on soil water potential. Hence, soil water potential appears to be responsible for microbial inhibition in overfertilized soils. Sites with coarse-textured soil may have limited ability to hold water, so increased fertilizer amendment may inhibit biodegradation (Braddock et al., 1997). Experiments conducted by Foght et al. (1999) showed that the addition of standard nutrient solution (containing soluble ammonium, nitrate and phosphate) stimulated aerobic degradation of saturates and *n*-alkanes. However, biodegradation of total aromatics and PAHs appeared to be inhibited due to the presence of ammonium. Degradation of the alkanes, stimulated by the addition of ammonium, resulted in significant acidification, which in turn inhibited aromatic degrading microorganisms. Swindoll et al. (1988) observed that the addition of inorganic nutrients to microcosms resulted in increased mineralization, whereas organic nutrient amendments increased lag time and resulted in less mineralization than unamended samples. This delay of metabolism was attributed to substrate inhibition by organic nutrients.

Nutrients are affected by other parameters including pH, geochemical concentrations, aquifer properties and temperature. Temperature often interacts with nutrient concentrations to influence microbial activity in contaminated soil (Braddock et al., 2001). Walworth and Reynolds (1995) observed that levels of petroleum decreased much more rapidly in soil incubated at 20°C than in 10°C soil. In addition, soil incubated at 20°C was greatly enhanced by the addition of P, whereas there was minimal effect at 10°C. Further influences of temperature are discussed in Section 3.5.

3.5 EFFECTS OF TEMPERATURE

Microbially mediated biodegradation reactions are strongly influenced by temperature. Cold temperatures affect chemical thermodynamics of a reaction, the reaction rate and microbial metabolism.

3.5.1 TEMPERATURE INFLUENCE ON THERMODYNAMICS

The equilibrium constant, K, is the ratio of product to reactant concentrations for a chemical reaction at specified standard conditions. The K value of each reaction shows the degree of biodegradation under standard conditions. A thermodynamic basis for predicting the change in equilibrium constant with temperature is described as (Sawyer et al., 1994):

$$\frac{d\ln K}{dT}=\frac{\Delta H^0}{RT^2} \qquad [12]$$

$d\ln K/dT$ = change in the natural log of the equilibrium constant with temperature

ΔH^0 = Change in Enthalpy at a specified temperature [kJ/mol]

R = Universal Gas constant [8.314 J/K mol]

T = Room temperature [298 K]

Under exothermic conditions the equilibrium constant will decrease with increasing temperature and for endothermic conditions, K increases (Sawyer et al., 1994). For narrow temperature ranges, ΔH^0 can be held constant and the integrated reaction is:

$$\ln\frac{K_{T_1}}{K_{T_2}}=-\frac{\Delta H^0}{R}\left(\frac{T_1-T_2}{T_1T_2}\right) \qquad [13]$$

K_1 = equilibrium constant at temperature, T_1

K_2 = equilibrium constant at temperature, T_2

3.5.2 TEMPERATURE INFLUENCE ON REACTION KINETICS

The rates of most chemical and biological reactions increase with temperature (Sawyer et al., 1994). The Arrhenius equation mathematically expresses the change in rate constant with temperature (Sawyer et al., 1994):

$$\frac{d(\ln k)}{dT}=\frac{E_a}{RT^2} \qquad [14]$$

$d(\ln k)/dT$ = change in the natural log of the rate constant with temperature

E_a = activation energy [J/mole]

This equation can be integrated between the limits of T_1 and T_2 (Sawyer et al., 1994):

$$\ln\frac{k_2}{k_1} = \frac{E_a(T_2 - T_1)}{RT_2T_1} \quad [15]$$

k_1 and k_2 = the rate constants at temperatures T_1 and T_2

If the process or reaction of concern operates over a narrow temperature range near ambient temperatures, the product of T_1 and T_2 is relatively constant. Thus, E_a/RT_2T_1 is substituted with a constant θ, and the temperature dependence of reaction rates can be expressed as:

$$\ln\frac{k_2}{k_1} = \theta(T_2 - T_1) \quad \text{or} \quad [16]$$

$$k_2 = k_1\theta^{(T_2-T_1)} \quad [17]$$

Values of θ for different reactions are shown in Table 3-5.

3.5.3 TEMPERATURE INFLUENCE ON BIODEGRADATION RATES

Biochemical reactions generally follow the van't Hoff rule of doubling reaction rate for every 10°C increase in temperature, over a limited temperature range (Sawyer et al., 1994). The response of biological and/or biochemical reactions to temperature can be quantitatively expressed as a Q_{10} value, which is the ratio of the reaction rate at a particular temperature to the rate 10°C lower (Yeung et al., 1997):

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{10/\Delta T} \quad [18]$$

ΔT = change in temperature from T_2 to T_1

The Q_{10} values can be related to θ (Section 3.5.2) by:

$$Q_{10} = \theta^{10} \qquad [19]$$

Theoretically, doubling the reaction rate for a 10°C increase should give a Q_{10} of 2 and θ of 1.072. Values of Q_{10} are listed in Table 3-5.

Table 3-5. Typical Arrhenius and Q_{10} Constants for Various Processes

Process	Q_{10}	θ	Temperature Range (°C)
[1]Activated Sludge	2.85		10-20
[1]Activated Sludge	2.22		15-25
[1]Activated Sludge	1.89		20-30
[1]Anaerobic Sludge	1.67		10-20
[1]Anaerobic Sludge	1.73		15-25
[1]Anaerobic Sludge	1.67		20-30
[1]Anaerobic Sludge	1.48		25-35
[1]Anaerobic Sludge	1.0		30-40
[2]Activated Sludge		1.00-1.04	
[2]Aerated Lagoons		1.06-1.12	
[3]Bioreactor	2.4 ± 0.2		5-20
[3]Bioreactor	1.4 ± 0.2		20-30
[4]Biodegradation of crude oil in Beach Gravel	3.3		6-16
[4]Biodegradation of crude oil in Beach Gravel	2.05		11-21

1 - Sawyer et al. (1994)
2 - Tchobanoglous (1979)
3 - Yeung et al. (1997)
4 - Gibbs et al. (1975)

3.5.4 TEMPERATURE INFLUENCE ON MICROBIAL BIODEGRADATION

The rate of metabolism of a microbial community is typically reduced by half for every 10°C reduction in temperature below the optimum temperature (Bradley and Chapelle, 1995). Microbial activity in groundwater is expected to be optimal between 20 and 40°C, and lower temperatures are assumed to significantly decrease activity. However, these assumptions do not take into account the ability of microorganisms to adapt to low temperatures (Bradley and Chapelle, 1995). Adaptation to low temperatures in groundwater is even more likely because seasonal fluctuation in temperature is low (Bradley and Chapelle, 1995).

Biodegradation of petroleum hydrocarbons at cold temperatures has been reported in a variety of soil, water and marine systems in the Arctic, Antarctic and alpine ecosystems. Cold-adapted microorganisms are essential components of the decontamination of hydrocarbon pollution in cold climates. The study site being examined is not in one of the aforementioned cold regions, however there are persistently low temperatures in the groundwater (~8°C). The optimum temperature for the biodegradation of hydrocarbons has generally been found to be in the range of 20-30°C (Atlas and Bartha, 1992). However, cold conditions can favour growth of microorganisms that are adapted to lower optimum temperatures. For example, Margesin and Schinner (1997b) found similar decontamination rates at 10°C and 25°C, which reflects the adaptation of indigenous soil microorganisms to cold temperatures.

Psychrotolerant microorganisms can adapt to low temperatures. Microbial adjustment to cold environments include the molecular adaptation of membrane lipid composition, protein synthesis and enzyme activity. Whyte et al. (1999) observed a specific strain of bacteria adapted to growth at a low temperature by decreasing the degree of saturation of membrane fatty acids. The bacteria appeared to adjust membrane fluidity in response to temperature as well as hydrocarbon toxicity.

At low temperatures, oil viscosity increases, there is reduced volatilization of short-chain alkanes and small aromatics that may be toxic, and water solubility increases. All these factors can delay and reduce microbial biodegradation (Margesin and Schinner, 1999). Temperature can also indirectly alter the pattern of hydrocarbon utilization. Cold temperatures can cause the precipitation of certain alkanes as waxes, thus diminishing their availability to the hydrocarbon degrading microorganisms (Margesin and Schinner, 1999).

3.6 EFFECT OF LIGHT NON-AQUEOUS PHASE LIQUID FREE-PHASE

The chemical phase of organic compounds determines whether they are available for biodegradation. PHCs such as gasoline, diesel and motor oil in the form of LNAPL are less readily degradable by microorganisms than those dissolved in water or sorbed to soil (US EPA, 1999). LNAPL also may have toxic effects on microorganisms.

The mass transfer of LNAPL to the aqueous phase is negligible over the short term, thus it may take tens or hundreds of years for degradation to be complete (Wiedemeier et al., 1999). Free-product present in the subsurface acts as a continuing source of contamination (Kampbell et al., 1996). The mass transfer from LNAPL-saturated soils or lenses floating on the water table will limit contaminant removal (Van Cauwenberghe and Roote, 1998). Consequently, the presence of free-phase LNAPL has the potential to slow down or even stop the biodegradation process. In addition, water table fluctuations caused by seasonal recharge or local pumping can create a smear zone that can increase the area of contamination (Parsons Engineering Science, 1999b).

3.7 UPSTREAM OIL AND GAS SITES

The sites under investigation in this study are mainly at upstream oil and gas sites. Upstream sites are defined from the point from where oil and gas leave the ground until they reach the refinery. Thus they include well sites, batteries, satellites, gas plants, pipelines, extraction plants, sour gas plants and a variety of other facilities. Previous studies have focused primarily on contamination at downstream sites (*i.e.* post-refining) such as underground storage tanks and service stations. PHCs at downstream sites are usually refined, whereas at upstream sites the contamination consists of unprocessed PHCs and may include other co-contaminants that may have an adverse effect on natural attenuation. Co-contaminants may include inorganic chemicals such as salts derived from produced water and anthropogenic nitrate, and process chemicals containing non-petroleum hydrocarbons such as glycol, amines, tetrahydrothiophene sulfolane (sulfolane) and diisopropanolamine (DIPA).

3.7.1 CAUSE OF SUBSURFACE CONTAMINATION

Groundwater contamination at upstream sites result from a variety of incidents. This includes leaking tanks, spills, pipeline leaks, well blowouts, seepage from flare pits, landfills or ponds, fallout from flare stacks, runoff from irrigation, and overflow of tanks, ponds and waster treatment facilities.

3.7.2 EFFECTS OF CO-CONTAMINANTS

Preferential degradation of non-petroleum hydrocarbons (non-PHCs) and co-contaminants is a concern when evaluating natural attenuation of PHCs at a site. For example, Corseuil et al. (2000) found that the presence of ethanol in a gasoline plume (from ethanol/gas mixtures at retail outlets) interfered with biodegradation of BTEX components. Ethanol appeared to have been preferentially degraded under aerobic and anaerobic conditions in the subsurface. Thus, available electron acceptors were depleted which subsequently impeded or retarded the biodegradation rate of BTEX.

Sulfolane and DIPA are common co-contaminants at upstream gas sites that may affect biodegradation of PHCs. Sulfolane, a physical solvent and DIPA, a chemical solvent are used in the Shell Sulfinol® process to remove hydrogen sulfide from natural gas (Greene et al., 1998). This process is necessary because the natural gas in western Canada is often sour, containing up to 35% hydrogen sulfide (Gieg et al., 1999a). PHC plumes at sour oil and gas facilities often contain sulfolane and/or DIPA due to commingling during disposal. Spills, leaky landfills and evaporation ponds at sour gas treatment plants containing sulfolane and DIPA (and PHCs) have contributed to subsurface contamination (Greene et al., 1998; Gieg et al., 1998). There have been no studies that examine the effect of these solvents on PHC natural attenuation. However, sulfolane and DIPA biodegradation appear to occur under aerobic and anaerobic conditions in the laboratory (Greene et al., 1998; Gieg et al., 1998; Gieg et al., 1999a). There are currently no studies that have examined *in-situ* biodegradation. These solvents may be preferentially degraded in the presence of PHCs, which could retard degradation of PHC contaminants.

Oil and gas production involves a dehydrating process that removes associated water. This water is often saline depending on depth and depositional geology of the oil and/or gas. The removal of water is usually conducted at the surface so leaks or spills are likely to contain saline water. However, refined or processed oil and gas has almost no salt associated because removal occurs upstream. Disposal of salt water can occur with other wastes, and thus leaks from flare pits and wastewater ponds often contain elevated chloride concentrations. Additionally salt water has a greater tendency to corrode, creating a greater potential for spills and leaks from tanks and pipelines. The presence of produced water containing chloride may adversely affect PHC biodegradation. Chloride is recalcitrant to degradation and is often used as a tracer in contaminant plumes to get an indication of plume migration and length. High salinity can inhibit microbial degradation of hydrocarbons in soil and groundwater due to changes in osmotic potential (Braddock et al., 1997).

3.7.3 DIESEL CONTAMINATION

Diesel fuel contamination at upstream and downstream sites can be attributed to spills and leaks from vehicles, drilling mud, tanks and other operational practices. The characteristic components of diesel fuel include *n*-alkanes, (n-C_9 to n-C_{27}), paraffinic (saturated hydrocarbons), aromatic and olefinic (unsaturated hydrocarbons) hydrocarbons (Table 1-2) (Bregnard et al., 1996). At the US EPA Superfund projects, diesel is the second most frequently treated type of contamination after benzene (Zytner et al., 2001).

Aliphatic compounds such as *n*-alkanes are a major component of diesel fuel, however due to very low aqueous solubility in water only a small fraction dissolves in water (Chapelle, 2001). On the other hand, BTEX compounds have significantly higher water solubility and may account for most of the dissolved fraction in water despite comprising less than 3% (by weight) of the diesel (Chapelle, 2001). Solubility differences affect the migration and biodegradation of PHCs in the groundwater. Therefore, site-specific laboratory studies using indigenous subsurface microorganisms may be necessary to examine the biodegradation rate of diesel components and determine if the rate of degradation will prevent the plume from migrating downstream. It is also important to determine the components of diesel contamination, as the original mixture may change over time due to weathering and degradation.

The relative biodegradability of diesel fuel components generally follows the order of *n*-alkanes > branched and cyclic alkanes>aromatics (Olson et al., 1999). There may be exceptions to this generalization, depending on environmental conditions. The aromatic fraction of diesel appears to biodegrade at a significantly slower rate in comparison to the aliphatic fraction (Olson et al., 1999). On the other hand, Foght et al. (1990) found that a mixed microbial population from a marine water-column mineralized phenanthrene and naphthalene faster than hexadecane. De Jonge et al. (1997) also observed preferential degradation of *n*-alkanes in fuel oil. Biodegradation rates appeared to increase with decreasing number of carbons. This is likely because shorter chain alkanes have higher water solubility and consequently are more available for microbial attack. However, as the concentration of fuel oil increases bioavailability is not as influenced by aqueous solubility of compounds. At higher concentrations, oil may be present as NAPL in the form of a film or dispersed droplets. Bacteria at the NAPL-water interface are able to degrade hydrocarbons that are relatively insoluble (de Jonge et al., 1997).

Gasoline is composed mainly of low to moderate weight compounds (C_3-C_{12}), while diesel contains a greater portion of higher molecular weight compounds (C_{10}-C_{30}) (Riser-Roberts, 1992). Compounds with higher molecular weights tend to be more resistant to biodegradation. Complete diesel degradation will likely be slower than BTEX because of the presence of heavier hydrocarbons which have a low aqueous solubility and are not readily bioavailable (Borden, 1994). Margesin and Schinner (1997a) noted that low degradation rates of diesel can be attributed to low bioavailability of the contaminant, accumulation of recalcitrant components, and lack of electron acceptors and nutrients required for cell growth.

Bregnard et al. (1996) conducted a study on the degradation of weathered diesel fuel and the effects of aerobic and nitrate reducing conditions. Significant degradation of the weathered diesel fuel took place under aerobic conditions, and only small amounts of diesel were recovered. However under denitrifying conditions the degradation was less pronounced as diesel was only reduced to 50% of the original concentration. Experiments with ^{14}C labelled toluene, naphthalene and hexadecane were used to conduct a carbon balance in the denitrifying microcosms at 12°C. The substrate carbon evolved as $^{14}CO_2$ was 53% for toluene, 43.9% for naphthalene and 4.7% for hexadecane. The remaining ^{14}C labelled substrate was recovered in the aquifer material and the butyl rubber stoppers.

Other terminal electron-accepting microorganisms are capable of biodegrading diesel under anaerobic conditions. After 201 days of incubation Caldwell et al. (1998) observed complete *n*-alkane (C_{15}-C_{34}) biodegradation to CO_2 under anaerobic sulfate-reducing conditions. Coates et al. (1997) also noted biodegradation of *n*-alkanes (C_{15}-C_{23}) under sulfate reducing conditions. Furthermore, sulfate-reducing bacteria capable of degrading *n*-alkanes have been isolated in a variety of studies (Aeckersberg et al., 1991; Rueter et al., 1994; So and Young, 1999a; So and Young, 1999b).

3.8 PLUME-A-THONS

Over the past decade a number of studies examining generic plume characteristics have been compiled into databases, then analyzed for evidence of characteristic responses, behaviours or potential correlations (Komex International Ltd., 2001b). These studies, also known as plume-a-thons, have provided insight into plume behaviour and support the use of natural attenuation as a remediation methodology at many hydrocarbon contaminated sites (Wiedemeier et al., 1999). These data provide information for predicting plume behaviour in future site investigations. A summary of relevant plume-a-thons that represent downstream site assessment are presented below.

3.8.1 CALIFORNIA LEAKING UNDERGROUND FUEL TANK (LUFT) HISTORICAL CASE ANALYSIS

The California Leaking Underground Fuel Tank (LUFT) Historical Case Analysis (also known as Lawrence Livermore National Laboratory (LLNL) Study) involved compiling and analysing plume data from 271 LUFT sites. The objectives of the analysis were to determine if PHC plumes behave in predictable ways, to identify the factors that influence the length and mass of hydrocarbon plumes, and to find the impact of PHC plumes on California's groundwater resources (Rice et al., 1995). Benzene concentration and trends were the focus of analysis in this study. Benzene plume length tended to change slowly and stabilize at relatively short distances from the PHC source. About 90% of plumes were less than 250 ft (76.2 m) and the median plume length was 101 ft (30.8 m). Plume length tended to change slowly over time, whereas benzene concentrations within the plume decreased much more rapidly. Hydrogeological parameters such as hydraulic conductivity, groundwater depth and gradient appeared to have little correlation with plume length. There are few tentative conclusions as to what factors influence the length and mass of PHC plumes. Of 271 plumes, 59% were stable, 33% were shrinking and only 8% were growing. The data also indicated that although active remediation may help reduce benzene concentrations, significant contaminant reduction can occur with passive remediation (Rice et al., 1995).

3.8.2 TEXAS BUREAU OF ECONOMIC GEOLOGY (BEG)

The Texas Bureau of Economic Geology (BEG) evaluated groundwater impacts from 217 sites in Texas where underground leaks have released fuel hydrocarbons. Site, soil, hydrogeological and chemical analytical data were compiled and entered into a database. The objectives were to determine dimensions of benzene plumes, predict plume concentrations and lengths, calculate biodegradation rates and classify plume behaviour to assist in exposure assessments. Most benzene plumes (75%) were less than 250 ft (76.2 m) long and were either stabilized or decreasing in length and concentration. Even without remediation, plume mass appeared to stabilize eventually and decline over time

(Mace et al., 1997). Thus, declines in plume mass and length result from natural attenuation processes or remediation activities or both. However, the length of a benzene plume could not be predicted on the basis of site hydrogeology or previous engineered remediation (Mace et al., 1997). There was also no statistical difference in benzene plume length between plumes in differing hydrogeological areas. Factors such as the amount of fuel from the source and the effectiveness of natural attenuation may exert greater influence on plume length than hydrogeology or active remediation.

3.8.3 FLORIDA LEAKING UNDERGROUND STORAGE TANK (LUST) SITES

A Florida risk-based corrective action (RBCA) study (Groundwater Services, 1997) conducted a technical evaluation of the impact of risk-based corrective action policy issues on the cost of corrective measures at leaking underground storage tanks (LUST) sites in the state of Florida. The purpose of the study was to collect and analyze data from 117 LUST sites with PHC releases, and use the compiled information to aid in implementing RBCA policies for the cleanup of storage tanks (Groundwater Services, 1997). Plumes that were delineated to a 50 µg/L benzene limit (51 sites) had a median length of 90 ft (27.4 m), whereas plumes delineated to a 1 µg /L limit (21 sites) had a median plume length of 120 ft (36.6 m) (Newell and Connor, 1998). Also, 51% of the sites had undergone or were currently being subjected to active groundwater remediation, such as soil removal, SVE, free-product recovery and containment.

3.8.4 HYDROGEOLOGICAL DATABASE (HGDB)

The Hydrogeological Database (HGDB) (Newell and Connor, 1998) contains hydrogeological and chemical information from 400 site investigations across the United States. This database includes groundwater plume dimensions for various contaminants, including 42 service station BTEX sites, 40 non-service station BTEX sites, 78 chlorinated ethene sites, 25 non-ethene solvent sites, and 21 inorganic sites. The 42 service station plumes had a median plume length of 213 ft (64.9 m). Newell et al. (1990) found that BTEX plumes tended to be much smaller than other types of plumes. This is likely due to a smaller source zone area and the more biodegradable nature of BTEX relative to other contaminants.

Data from the HGDB were reanalyzed along with the above-mentioned studies by Newell and Connor (1998) who found that most hydrocarbon plumes associated with leaking fuel tanks at service stations were under 200 ft (61 m) long. Nearly 75% of plumes were found to be either stable or shrinking, however plume concentrations were predominantly decreasing (47-59%), whereas plume lengths were usually stable (42-61%). Results also suggested that dissolved hydrocarbon plumes tended to reduce more rapidly in concentration than length. The compiled data from the California and Texas studies (Rice et al., 1995; Mace et al., 1997) found that once a dissolved BTEX plume begins to shrink, the rate of decline in plume mass is rapid. Assuming first order decay, a shrinking plume appears to require 5 to 10 years for the BTEX concentration to drop from 1 ppm to 1 ppb. The studies from Texas, California and Florida (Rice et al., 1995; Groundwater Services, 1997; Mace et al., 1997) concluded that active soil and groundwater remediation do not necessarily result in smaller BTEX plumes, indicating that natural attenuation can often be used as a cost-effective remediation option.

3.8.5 U.S. AIR FORCE STUDY

Data were compiled from 38 Air Force sites including detailed information on plume characteristics, electron acceptors, and general site conditions present at PHC sites (Wiedemeier et al., 1999). The data from this study are likely to be of higher quality compared to previously mentioned studies because the median number of wells was 25 compared to 5-6 in the Rice et al. (1995) and Mace et al. (1997) studies, the sites were better monitored and geochemical data were collected so that biodegradation capacity could be calculated. The information gathered in the Air Force study allows a thorough comparison to be made with the database developed for Alberta oil and gas sites. This study is discussed in detail in the next section.

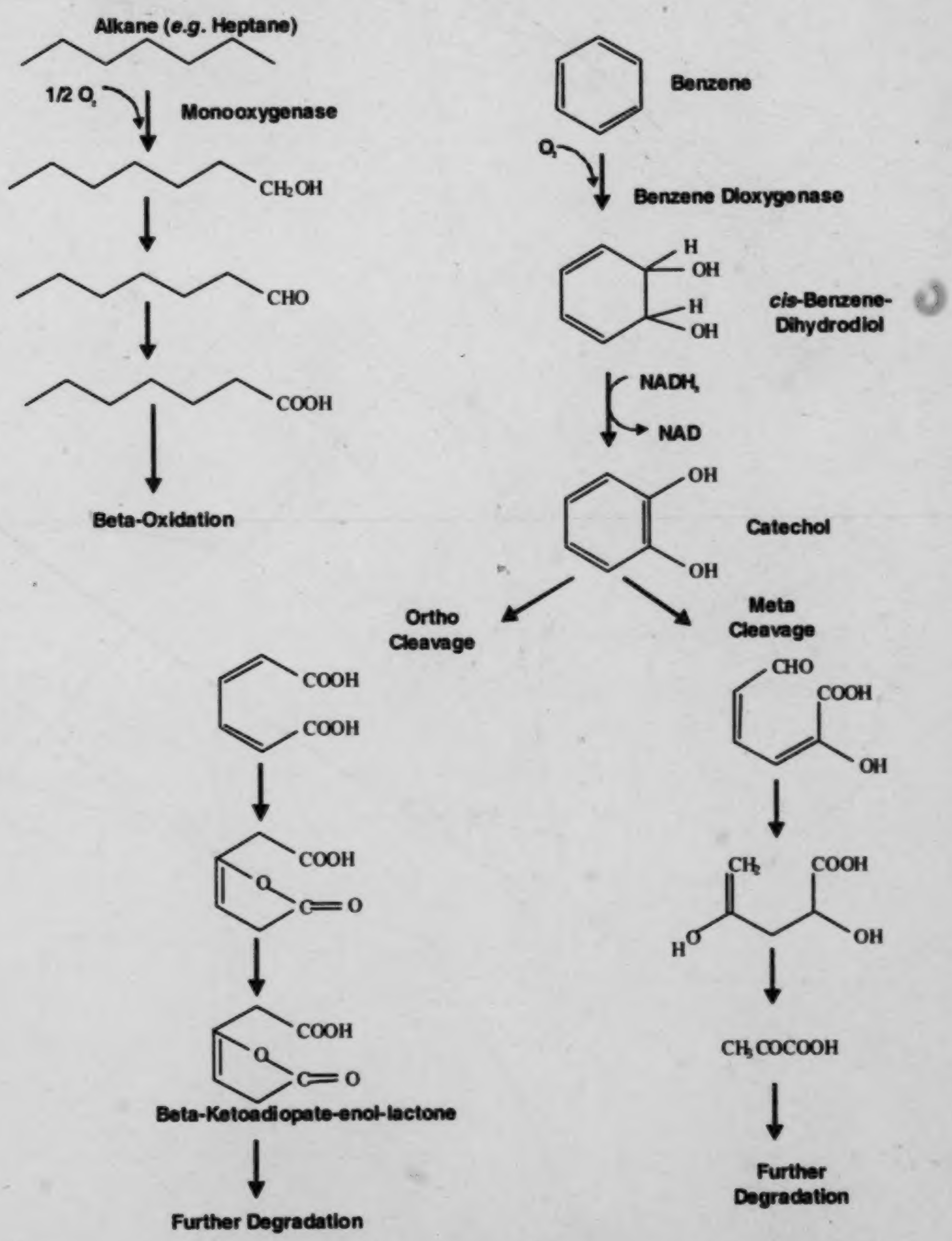

Alkane (*e.g.* Heptane)
1/2 O₂
Monooxygenase
CH₂OH
CHO
COOH
Beta-Oxidation
Benzene
O₂
Benzene Dioxygenase
H
OH
H
OH
cis-Benzene-Dihydrodiol
NADH,
NAD
OH
Catechol
OH
Ortho Cleavage
Meta Cleavage
COOH
COOH
CHO
COOH
OH
COOH
O
C=O
CH₂
COOH
HO
OH
COOH
O
C=O
Beta-Ketoadiopate-enol-lactone
CH₃COCOOH
Further Degradation
Further Degradation

Figure 3-1. Possible Pathways of Hydrocarbon Degradation by Aerobic Microorganisms (Heider et al., 1999).

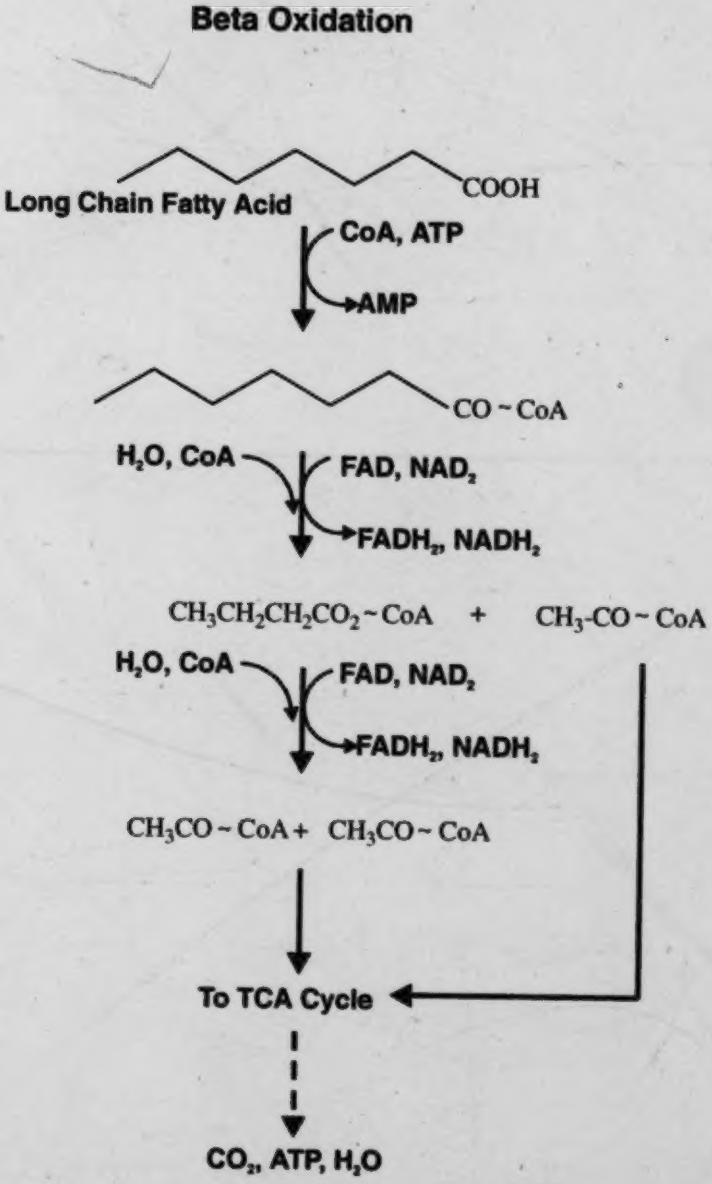

Beta Oxidation
Long Chain Fatty Acid
COOH
CoA, ATP
AMP
CO ~ CoA
H₂O, CoA
FAD, NAD₂
FADH₂, NADH₂
CH₃CH₂CH₂CO₂ ~ CoA + CH₃-CO ~ CoA
H₂O, CoA
FAD, NAD₂
FADH₂, NADH₂
CH₃CO ~ CoA + CH₃CO ~ CoA
To TCA Cycle
CO₂, ATP, H₂O

Figure 3-2. Beta-Oxidation of Long-Chain Fatty Acids (Chapelle, 2001).

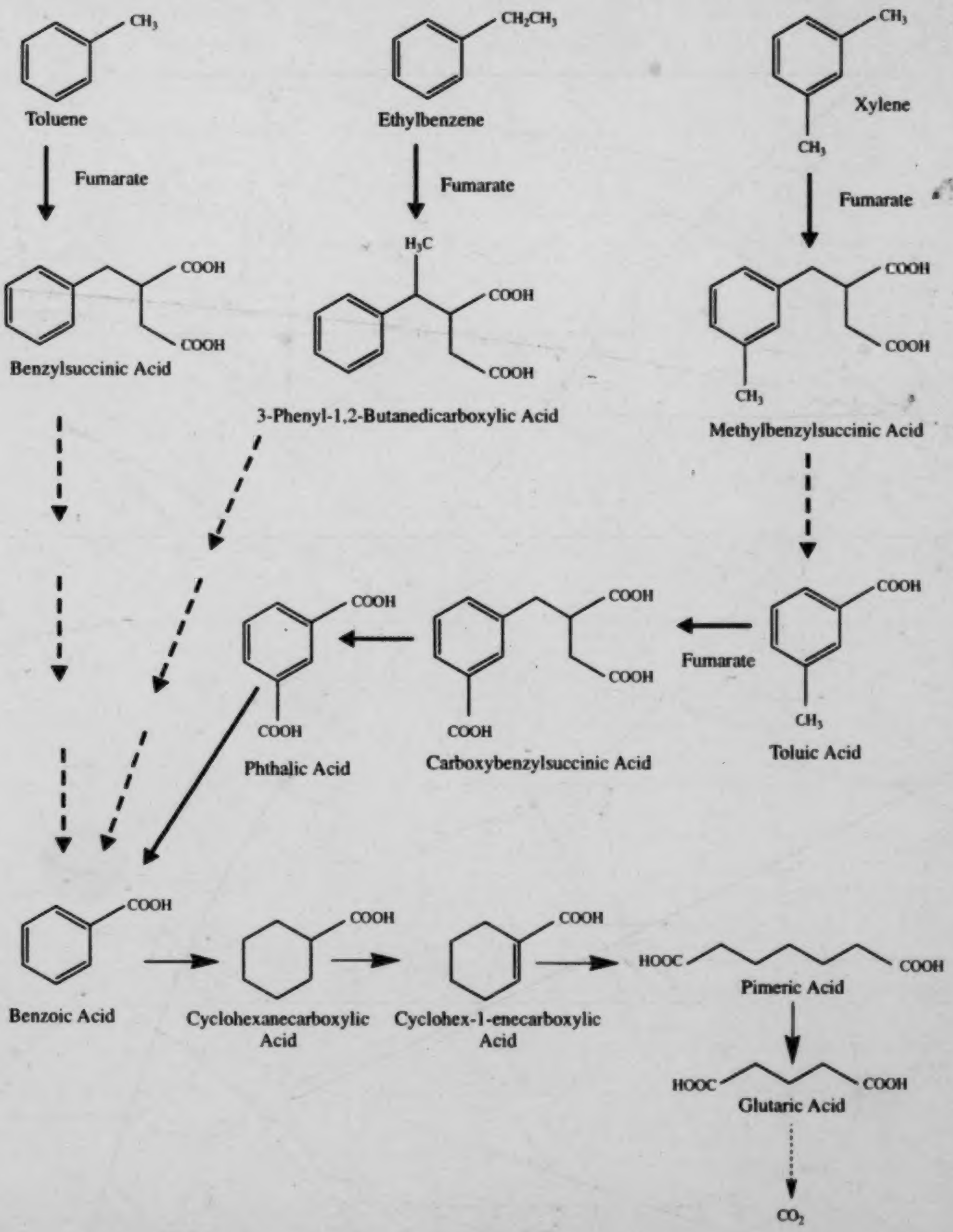

CH3
Toluene
Fumarate
CH2CH3
Ethylbenzene
Fumarate
CH3
Xylene
CH3
Fumarate
COOH
COOH
Benzylsuccinic Acid
H3C
COOH
COOH
3-Phenyl-1,2-Butanedicarboxylic Acid
COOH
COOH
CH3
Methylbenzylsuccinic Acid
COOH
COOH
Phthalic Acid
COOH
COOH
COOH
Carboxybenzylsuccinic Acid
Fumarate
COOH
CH3
Toluic Acid
COOH
Benzoic Acid
COOH
Cyclohexanecarboxylic Acid
COOH
Cyclohex-1-enecarboxylic Acid
HOOC
COOH
Pimeric Acid
HOOC
COOH
Glutaric Acid
CO2

Figure 3-3. Proposed Pathways for Degradation of Toluene, Xylene, Ethylbenzene, Toluic Acid and Benzoic Acid under Anaerobic Conditions (Elshahed et al., 2001).

4. PLUME-A-THON

4.1 INTRODUCTION

This chapter was developed in conjunction with Komex International Ltd. The information presented was originally written in a Komex report titled, "Assessment of Monitored Natural Attenuation at Alberta Oil and Gas Facilities" prepared for Canadian Association of Petroleum Producers, Alberta Environment and Environment Canada. James Armstrong was a significant contributor to this report.

4.2 METHODOLOGY

4.2.1 DATA SOURCES

The data were extracted from information contained in Komex reports and files stored in Calgary. The CORONA database mainly comprises Alberta sites, but includes several sites from British Columbia and Saskatchewan.

Previous 'plume-a-thon' studies focused on contamination at downstream sites such as underground storage tanks and service stations. PHCs at these sites are usually refined, whereas at upstream sites the contamination consists of unprocessed PHCs and other co-contaminants including process chemicals that may have an adverse affect on natural attenuation. Co-contaminants include inorganics such as chloride and nitrate derived from produced water, and process chemicals containing non-PHCs such as glycol, amines, sulfolane and DIPA.

The database contains historical groundwater monitoring data from 125 contaminated sites. Hydrocarbon contamination was present at 101 of these sites, defined as the presence of petroleum hydrocarbons such as BTEX, TPH and TEH. The remaining 24 sites contain non-PHC contaminants and /or inorganic compounds.

4.2.2 DATABASE STRUCTURE AND DEVELOPMENT

A basic database template was constructed in Microsoft Access© to store the plume-a-thon data. This template was then refined as needed to incorporate new types of data as they were encountered when entering data from various sites. Modifications to the database were also made to investigate potential new relationships that became evident during the data entry process. Finally, the completed database was checked for accuracy and repeatability of interpretation. During trend assessments, any outlying or suspect values were re-checked for validity and accuracy. The present format can be modified further if necessary to allow for new fields and/or other applications.

The database was developed using available site characterization information, but contaminant conditions were derived only from groundwater monitoring programs. No data are included for any soil, rock or sediment samples analyzed. This decision was made at the start of the study for two reasons. Firstly, groundwater is one of the two main transport pathways for contaminants. The other main potential pathway (vapour transport) has limited available site monitoring data. Secondly, a relatively comprehensive groundwater data set was available for the large number of sites considered in this study. Based on this selection of groundwater as the principal transport mechanism, categories and parameters were selected to identify sites where natural attenuation might be limiting plume growth.

The data for each plume were entered into a form. Figure 4-1 shows the cover page for each individual plume. The plume ID number consists of the Komex job number followed by the plume number. For example for 37270700.1, 37270700 is the job number and .1 is the first plume being characterized at this specific site. Therefore, data can be entered for one or more plumes at any given site.

The characterization details for each plume are entered into six separate sections of the form shown as tabs in Figure 4-1:

1. Monitoring Site and Time - general information about the plume, including site identifier, length of site monitoring history, the number and location of monitoring wells, and site geology;

2. Groundwater characteristics - details on site hydrogeology, including hydraulic conductivity, depth of the water table and groundwater velocity;

3. Contaminant concentrations - contaminant name(s) and temporal concentration changes over the monitoring period for each contaminant in the plume;

4. Plume characteristics - plume source and type of release, plume geometry (length and width) and overall plume trend (stable, shrinking, growing or indeterminate) for each contaminant identified in the plume;

5. Geochemical indicators - Concentration (plume and background) and temporal changes in dissolved oxygen, nitrate, iron, manganese, sulfate and methane; and,

6. Plume remediation - summary of engineered remediation actions and comments about the plume and the site.

Each section of the database contains a number of fields that are used to provide details for each individual plume. All words that are in **bold** in the following sections refer to parameters in the database. Each section has a **data quality assessment** field that evaluates the data either as good, acceptable, bad or unknown. This information was obtained from project managers. Each section of the database and the method of input are described in the following sections.

4.2.2.1 Monitoring Site and Time

This page includes basic information that can be used to quickly identify the site where the plume is located in Western Canada. Figure 4-2 illustrates the monitoring site and time section as it appears in MS Access. The **project number** and **job number** refer to the identification code used by Komex International to identify each project at a site. The **plume** number is used to separate different plumes at the same site. The **name** of the site is entered as specified on the report from which all the data for each plume entry is gathered. The **LSD** is the legal sub division where the site is located. **Rock?** is checked if the groundwater plume is in bedrock and unchecked if the plume is in the surficial overburden such as clay, silt or sand. **Permeability** is generally classified based on geology type, which is divided into bedrock (sandstone, siltstone and shale) and surficial (sand, silt and clay) geology. Sandstone and sand were deemed to have high permeability, siltstone and silt medium permeability, and shale and clay have low permeability. Permeability and **geology type** are derived from the lithology within the plume area. **Geology type** is the lithology that is reported in the borehole logs, whereas permeability is a generalization. If more than one type of lithology was reported, then the hydraulic conductivity value given in the report was compared to values of various geology types given by Freeze and Cherry (1979), and the corresponding geology type was entered into the database.

The box titled **monitoring well list** contains the monitoring wells that were used to characterize each plume. There are four types of monitoring wells, these are labelled as: plume (to characterize), plume delineation (in the plume), plume edge delineation (non-detects) and background. Plume (to characterize) is the monitoring well that is used to characterize the maximum concentration of contaminants. This monitoring well is usually near the source of contamination. Plume delineation (in the plume) includes monitoring wells that are downgradient from the source, and were used to determine length, width and spatial trends. Plume edge delineation (non-detects) are monitoring wells that were beyond the edge of the plume that give an indication of the plume boundaries. Background monitoring wells are in a similar geologic unit and depth as plume (to characterize) monitoring wells. Background wells must have contained no contamination, and are supposed to represent background (ideally, upgradient) conditions prior to contamination.

The **monitoring period of site** refers to the years that the site has undergone groundwater monitoring either by Komex or another company. The **monitoring period of plume** refers to the time period that the plume has been monitored, and **number of years** gives the total amount of years the plume has been monitored. The **number of monitoring wells in area** is the total number of monitoring wells in the monitoring well list excluding any background wells. The **location of background monitoring well(s)** can either be upstream, cross-gradient or downstream from the plume. An upstream monitoring well is preferred because there is less likelihood of future contamination from the plume, the TEA concentration is most likely to represent conditions flowing into the source area, and there is less likelihood of unreported metabolic intermediates being represented. Finally, a checked box indicates a **petroleum hydrocarbon** plume.

4.2.2.2 Groundwater Characteristics

Local aquifer characteristics are included in this section (Figure 4-3). The first parameter is the **hydraulic conductivity** or K (m/s) of the monitoring wells in the plume area. Hydraulic conductivity values were usually obtained using slug tests. The **depth** (m) is measured from the ground surface to the water level in the monitoring well that is used to characterize the plume. The **gradient** or i at the plume site is calculated by dividing the change of the water table elevation by the distance over which the change occurs. The **velocity** (m/yr) of groundwater flow is calculated by:

$$v = \frac{Ki}{n} \times 3.15 \times 10^{7}\ \text{s/yr} \qquad \textbf{[20]}$$

where v is velocity (m/yr), K is hydraulic conductivity (m/s), i is the gradient, and n is porosity. The porosity was determined from the geology type. If no porosity was reported a value of 0.1 was used to give a conservative estimate of growndwater velocity. The groundwater velocity was calculated when an average velocity had not been reported. In many reports the maximum hydraulic conductivity was used to calculate a maximum velocity. Depending on the distribution of hydraulic conductivity measured at a site, this value may be overly conservative. The **direction of groundwater flow** is the cardinal direction (N, NW, NE, etc.) of groundwater flow in the plume area.

4.2.2.3 Contaminants

This section contains the concentrations and trends of individual contaminants in each plume (Figure 4-4). **FP-Presence** or presence of free-product in the plume monitoring well can be reported as yes, no, sheen or NA (data is not applicable or available). If free product was present, the thickness in metres was entered into FP-Thickness. The temporal trend in free-product thickness (FP-Trend) is characterized by Down, Up, Steady, Indeterminate or NA. These identifiers were interpreted according to continuous trends, except:

- Indeterminate indicates the trend had no distinct pattern; and,
- NA means that there is no free product or insufficient data to determine a trend.

The specific contaminants that could be characterized in each plume include benzene (B), toluene (T), ethylbenzene (E), total xylenes (X), total purgeable hydrocarbons (TPH), total extractable hydrocarbons (TEH), chloride (Cl), dissolved Kjeldahl nitrogen (DKN), and dissolved organic carbon (DOC). Any other contaminants are called **other 1-5**. In the **presence** field of each contaminant the entry is either present, absent or NA (if there is no information). If the contaminant is not one of the specific contaminants listed above, this field indicates the type of contamination. In the **start** parameter, the earliest reported concentration (mg/L) of the contaminant at the plume monitoring well is recorded, except as noted. If the contaminant has a stable temporal trend the approximate average is entered, and if the plume has an indeterminate trend the highest concentration is reported. The **finish** field for all contaminants is the most recent measured concentration (mg/L), except as noted. If the contaminant has a stable trend then this field is left blank, whereas if the plume has an indeterminate trend then a minimum concentration is recorded. The **temporal** trend refers to how the concentration changes over time; the choices are down, up, indeterminate or NA (using a similar approach as described for the free-product trend). It should be noted that the start and finish concentrations were a guide and the important parameter is the temporal trend. To avoid future confusion, contaminants with an indeterminate trend should be left blank in the finish concentration field. The **spatial** trend has the same trend descriptors except refers to the change in concentration downstream from the source. If information on a contaminant was not included in any reports relating to the job number then NA must be entered into the appropriate field. For example, if there are no downstream monitoring wells then the spatial trend is NA.

4.2.2.4 Plume Characteristics

This section consists of information about the specific plume being characterized. Figure 4-5 shows the various parameters that are entered into this portion of the database. First, the type of **facility** is recorded. The facility is chosen from a list compiled from all the facilities investigated as shown in Table 4-1.

Table 4-1. Facilities in the NA Database

Type of Facility	Type of Facility
Battery	Pilot Plant
Central Treating and Waterflood Plant	Pipeline
Compressor Station	Production Complex
Cycling Plant	Refinery
Extraction Plant	Satellite
Fractionation Plant	Sour Water Plant
Gas Plant	Station
Landfarm	Wellsite
Other	

The **source** of the plume is where the contamination originated at the site. The choices (Table 4-2) were derived from the various reports reviewed.

Table 4-2. Contaminant Sources Included in NA Database

Type of Source	Type of Source
Barrel	Pipeline
Compressor	Pond
Fire Training Area	Process Area
Flare Pit	Recovery area
Flare Stack	Storage Area
Header	Sulfur Block
Inlet Area	Sulfur Prilling Area
Irrigation	Sump/Pit
Lagoon	Tank
Landfill	Waste Area
Loading Area	Well
Not Available	

The field labelled **description of source** allows specific information pertaining to the source of the plume to be recorded. If the source had been removed, then the **date of source removal** was entered. The **type** of contamination refers to how the plume occurred, whether it was a spill, leak, break, overflow or the information is not available (NA).

The next few fields deal with the type of contaminants and the size of the plume. There is often more than one type of contaminant present in the plume area and these co-contaminants may affect natural attenuation behaviour. **Which PHC?** is the type of petroleum hydrocarbon present, currently identified as BTEX (any of benzene, toluene, ethylbenzene or xylene), TPH or TEH. The **PHC length (m)** is the estimated length of the petroleum hydrocarbon plume. This is measured from the inferred or identified source of contamination to the edge of the plume where the contaminant is below the detection limit. The **PHC length qualifier** is used to record if the plume length is greater than (>), less than (<), or an approximation (?) of the measured plume length. This field is required to give further description and record any uncertainties of plume length. **PHC width (m)** is the estimated width of the petroleum hydrocarbon plume, and the **PHC width qualifier** is used to record any uncertainties. **Which non-PHC?** refers to contaminants that are not petroleum, such as process chemicals including sulfolane, DIPA, amines, glycols and phenols. Combinations of these chemicals can also be entered into this field. The **non-PHC length, width** and **qualifiers** are inserted in the same manner as PHC length, width and qualifiers. **Which inorganic?** Identifies any inorganic contaminants usually, but not limited to, one or more of chloride, sulfate, nitrate and metals. The **inorganic length**, **width** and **qualifiers** are entered as in the PHC and non-PHC fields.

The **end condition** explains how the plume terminates. The plume could either be exhausted, cut-off by a natural barrier like a stream, intercepted by an artificial barrier such as a trench or have an unknown end condition due to lack of information. The **age (yr)** of the plume was determined based on when the contamination occurred. Input for this field often had to be estimated. Chloride concentrations were often above background levels in conjunction with hydrocarbon contamination because the process water had a high salt content. Thus, chloride may be used as a **tracer** to determine how far contamination extends due solely to advection and dispersion. The overall plume **trend** takes all the contaminant trends in the previous section into consideration and classifies the plume as shrinking, stable, growing indeterminate or NA. A shrinking plume has a continuous decrease in contaminant concentration over time, a growing plume increases in concentration, a stable plume tends to have little variation in concentration with time, and an indeterminate plume has no distinct pattern and varies in concentration throughout the monitoring period. If there is lack of information, such as when the monitoring period has been less than a year, then the trend is labelled as NA. **Plume character** identifies whether the plume is discrete, continuous or unknown. A continuous plume appears to be constantly replenished by the source, so concentrations of contaminants are high near the source and decrease further away. A discrete plume means that the source may be removed or contained. The original source area is thus no longer contributing to the plume. A plume with an unknown character means there are probably not enough monitoring wells to delineate the plume and/or determine its character. **Plume type** identifies the type of contamination in the plume. Options include PHC, non-PHC, inorganic or any combination of the three (*i.e.* mixed - PHC and non-PHC).

4.2.2.5 Geochemical Indicators

This section records the concentrations and trends of geochemical indicators of NA at the monitoring well that are being used to characterize the plume (Figure 4-6). Changes in concentrations of the TEAs or microbial respiration by-products indicate the types of redox reactions that are occurring as well as the amount of biodegradation by microbes. As an example, the theoretical amount of degradation by aerobic microbes is estimated from the difference in dissolved oxygen between the plume and background areas. **Start** and **finish** concentrations (mg/L) of TEAs or by-products were recorded to determine whether they change over time. The dissolved oxygen **background** concentration (mg/L) was compared to the DO concentration in the plume to determine the EBC (Section 4.3.8) by aerobic microorganisms. The background concentration was taken from the background monitoring well, having similar geological conditions as the plume monitoring well(s). The EBC of denitrifying microorganisms was calculated by the depletion of nitrate in the plume. The start, finish and background concentrations for nitrate and other geochemical indicators were entered in the same manner as the dissolved oxygen parameters. EBC by iron and manganese reducing microorganisms was calculated using the reduction by-products, dissolved iron (Fe^{2+}) and dissolved manganese (Mn^{2+}). An increase in concentration of these compounds indicates the contribution of biodegradation by iron or manganese reducing microbes. Potential activity by sulfate-reducing bacteria was measured by depletion of sulfate in the plume compared to background concentrations. Methanogenic bacteria dominate as available sulfate is consumed. These bacteria use carbon dioxide as an electron acceptor and produce methane as a by-product. Dissolved methane is used to measure the degree of activity. The majority of sites and plumes investigated for this plume-a-thon did not have available methane data. This field is included for future use, if data for other sites include methane. The trend for all geochemical indicators was temporal and can be down, up, indeterminate or NA.

4.2.2.6 Engineered Remediation

The final section of the database summarizes any type of engineered remediation that may affect the natural attenuation process (Figure 4-7). The date when the remediation occurred was entered into the appropriate field. **Land farming** involves spreading and mixing the contaminated soil over a large area at the surface to stimulate aerobic biodegradation. **Excavation** means digging out and removing contaminated soil from the site. Excavation is normally the preferred solution for small volumes of easily accessible, contaminated soil. **Pump and treat** is the process of pumping the contaminated water to the surface and treating it at an above ground facility. **Soil Vapour Extraction (SVE)** is the process of extracting soil gas from the vadose zone to enhance the volatilization of contaminants and remove and treat contamination in the vapour-phase. This technology is effective for treatment of volatile contaminants such as gasoline and chlorinated solvents in the vadose zone (Downey et al., 1999). **Bioventing** is the process of injecting or extracting air from the vadose zone to supply oxygen for aerobic biodegradation of contaminants such as petroleum hydrocarbons. **Multiphase extraction (MPE)** is also known as dual-phase extraction or bioslurping. This technology combines free product recovery, SVE and bioventing and is particularly effective at reducing fuel residuals that are in the capillary fringe (Downey et al., 1999). **Trench and gate** involves intercepting the groundwater and funnelling it through a permeable media reactor to reduce contamination concentrations. **Other** types of contamination include low temperature thermal desorption, biopiles, biosparging, barrier/treatment walls and several others. New technologies are always being introduced so the list of engineered remediation will likely increase. There is also a **comment** box to add any extra information about the plume.

4.2.3 METHODOLOGY USED FOR ANALYSIS

The features of MS Access allow the database to be queried for specific parameters. For example, plume lengths and velocities could be extracted only for petroleum hydrocarbon plumes. The queried data were then exported into Excel and analyzed for trends. A number of queries were made to evaluate a variety of potential relationships, based on current understanding of natural attenuation processes. Relationships were broadly derived from, but not limited by, existing plume-a-thon studies. Efforts are ongoing to determine if a cluster analysis might identify relationships not identified from the direct correlation method used to date.

4.2.4 INFORMATION NOT AVAILABLE IN THE DATABASE

The database aims to summarize detailed information about individual plumes at specific sites. This approach means that influences from site heterogeneities may not be adequately captured by the analysis. This limitation is most likely to apply to discretely fractured systems, such as fractured bedrock.

The site selection approach filtered all potential sites by identifying those sites where petroleum hydrocarbon impact may have occurred. This approach may have missed a small number of sites, for example, where PHC analyses addressed only TEH. However, this is unlikely because of the strong regulatory controls on BTEX compounds. Sites with only inorganic compounds, such as chloride, were generally not included in the database. These plumes typically attenuate by dilution and/or sorption depending on hydrogeological conditions. Data for these plume types could eventually be entered into a similar database to examine these processes in Alberta.

4.3 RESULTS AND DISCUSSION OF PLUME-A-THON ANALYSIS

4.3.1 SUMMARY OF DATA

Files from a total of 253 sites were examined for entry into the plume-a-thon. These sites were initially selected from the Komex chemistry database by identifying all sites where benzene analysis was requested. This approach was used to include sites where organic contamination was an issue in the water supply. Analysis of benzene usually occurs when assessing subsurface impact associated with oil and gas facilities.

The 253 sites were then reviewed and selected for entry if they were relevant to NA investigations. Sites were not discarded if data did not support natural attenuation. This resulted in data from 125 sites being entered into the database and 128 sites being uncharacterized. The distribution of uncharacterized sites is summarized in Figure 4-8. Typical reasons for discarded sites included:

- Lack of information (*i.e.* one sampling visit showing no detectable PHCs)
- Information not applicable to NA (*i.e.* nearby resident drinking water well testing)
- No detectable contamination (*i.e.* all samples below detection limit)
- Only inorganic contamination (*i.e.* chloride, nitrate or sulfate contamination)
- Non-petroleum hydrocarbon compounds only, with insufficient evidence to assess natural attenuation (*i.e.* sulfolane, glycols)

There are entries in the data base that contain solely non-PHC plumes, however the information has not been developed to the same extent as plumes that contain PHCs. Since the plume-a-thon is an ongoing study, additional sites that have non-PHC contamination will eventually be included in the database to allow comparisons between natural attenuation of fuel hydrocarbons and other types of contaminants.

4.3.2 DISTRIBUTIONS OF SITES AND PLUMES

The 125 characterized sites had a combined total of 236 separate plumes (there are often multiple plumes at a site) entered into the database as distinct data sets. Sites and plumes were classified according to the various groundwater contaminants encountered (see Section 4.2.2.4 for explanation of plume types). The distributions of site and plume classifications are summarized in Table 4-3.

Table 4-3. Site and Plume Distribution

Sites (Total =125)	Plumes (Total =236)	Plume Classification	Percentage
PHC (101)	159	34 PHC only	14%
		95 PHC and inorganics	41%
		7 PHC and non-PHC	3%
		23 PHC, non-PHC, and inorganics	10%
Non-PHC (24)	77	60 inorganic only	25%
		17 inorganic and non-PHC	7%

Table 4-3 shows that approximately 60% of the reviewed plumes contain more than one class of contaminant, whereas only 14% of plumes have only PHC contamination. Considering just the 159 PHC plumes, approximately 79% of plumes contain multiple contaminants.

Sites that had any type of PHC contamination were classified as 'PHC sites' but they could contain additional plumes that did not have any hydrocarbon contamination (*i.e.* chloride or sulfolane). These non-PHC plumes were entered into the database but not characterized to the same extent as PHC plumes. This accounts for the relatively high number of non-PHC plumes (32%).

A wide range of contaminant combinations was identified in the plumes with no PHCs, as shown in Figure 4-9. This figure shows that almost all of the non-PHC plumes (71 of 77, 93%) contain chloride, while the remainder had either sulfate or nitrate contamination. Plumes with sulfolane, DIPA, glycol or phenols always include chloride as a co-contaminant. Chloride is commonly used as a contaminant indicator at upstream oil and gas sites.

4.3.3 PLUME TRENDS

Plumes were classified as either shrinking, stable, growing or indeterminate, depending on historical concentration trends. Indeterminate plumes had either insufficient data or too much variability to identify a concentration trend. The trends for hydrocarbon and non-hydrocarbon plumes are compared in Figure 4-10. This figure shows that nearly three-quarters of all hydrocarbon plumes were either stable (45%) or shrinking (25%), and only 10 plumes (6%) were growing. The majority of plumes identified as growing were often affected by increases in co-contaminants concentrations (*i.e.* chloride). In contrast, non-PHC plumes were found to be stable (50%) or growing (25%) over time. This is an indication of the biodegradable nature of fuel hydrocarbon constituents relative to other contaminants. Growing plumes usually contained chloride, which attenuates via dilution.

Results of other fuel site studies (with no co-contaminants) can be compared to the study. These studies consisted of downstream sites, whereas the CORONA database contains mainly upstream sites, thus the presence of process chemical and co-contaminants may effect the rate of attenuation. In the California LUFT analyses, Rice et al. (1995) found that 59.4% of benzene plumes had a decreasing concentration, 7.7% were increasing and 32.8% had no significant trend. Similarly the Texas BEG study by Mace et al. (1997) reported that half of the plumes were decreasing in plume mass, one-third were stable and only 14% were increasing in concentration.

There are a number of potential reasons for the comparatively lower number of decreasing PHC plumes in this study. First, biodegradation can be inhibited due to the presence of process chemicals and co-contaminants. Secondly, the biodegradation rates in Alberta are generally slower due to the cooler climate. Finally, there are limited data available to interpret trends at some sites.

4.3.4 CONTAMINANT TYPES AND SOURCES

The source of contamination was interpreted for 159 PHC plumes. More than 50% of the plumes appear to be from tanks, the process area or flare pits. Contamination causes were assessed based on available information, with results presented in Table 4-4.

Table 4-4. The Cause of Contamination for Hydrocarbon Plumes (Total = 159)

Plume Cause	Leak	Spill	Blowout	Overflow	Unknown
Occurrence (Total = 159)	108 (68%)	11 (7%)	3 (2%)	1 (<1%)	36 (23%)

The majority of plumes were caused due to leaks (68%). A relatively large portion (23%) of plumes are derived from unknown causes that may include spills, undetected leaks, former storage and daily operational practices at the site.

4.3.5 MONITORING COVERAGE (NUMBER OF MONITORING WELLS)

Database interpretation for evidence of NA depends on the level of monitoring detail. This factor was assessed by plotting the number of monitoring wells used to define each of the 159 hydrocarbon plumes (Figure 4-11). The median number of wells for each plume in the database is 3 to 4. Figure 4-11 shows that there are a large number of plumes with one monitoring well (41) and most plumes (62%) are characterized by fewer than five monitoring wells. Comparatively, the Air Force database by Wiedemeier et al. (1999) contained a median of 25 wells, and there were 5 to 6 wells in the California and Texas studies (Rice et al., 1995; Mace et al., 1997).

The lack of coverage may introduce some uncertainty in data analysis because of limited data available for characterizing plume geometry, spatial variations in plume concentrations and detailed assessment of local groundwater geochemistry and flow conditions. It is also difficult to determine whether the contamination is only in the immediate area of the monitoring well or if it extends further downstream.

4.3.6 BTEX DISTRIBUTIONS, CORRELATIONS AND TRENDS

The following analysis examines the distribution of BTEX concentrations, the relationship between BTEX and other contaminants and the effect of BTEX degradation on plume trends. The distribution of BTEX concentrations at the hydrocarbon sites is summarized as percentiles in Table 4-5. A large number of sites have total BTEX concentrations less than 10 mg/L. The median concentration of BTEX was 2.42 mg/L, with values ranging from <0.0009 to 370.1 mg/L. It is possible that the very high BTEX concentrations are not just dissolved contaminants, but may include traces of free hydrocarbon. Table 4-5 emphasizes how the site data are skewed to low BTEX concentrations. In comparison, the Air Force sites have a median total BTEX concentration of 13 mg/L, a maximum of 405 mg/L and a minimum of <1mg/L (Wiedemeier et al., 1999).

Table 4-5. Summary of Distribution of BTEX Concentrations for PHC Plumes (mg/L) (Total = 159 Plumes).

Plume Initial Concentration (mg/L)	TEH	TPH	BTEX	B	T	E	X
No Data	76	18	5	6	7	9	6
Non Detect	19	19	10	9	8	9	9
0-0.99	19	30	57	78	92	119	84
1-9.9	17	42	47	45	30	21	39
10-99	15	42	36	19	21	1	21
>99	13	8	4	2	1	0	0

Minimum	<0.0009
10th Percentile	0.042
25th Percentile	0.20
Median	2.42
75th Percentile	13.88
90th Percentile	42.83
Maximum	370.1

PHC concentrations were examined for evidence of other potential compounds besides BTEX. Figure 4-12 illustrates the correlation between Total Purgeable Hydrocarbons (TPH – reported as C_3-C_{10} or C_3-C_{12}) and BTEX. The relative contribution of BTEX to TPH can be indicated by how far the total BTEX mass deviates from TPH. Theoretically, total BTEX should not exceed TPH (indicated by a line with a slope of 1 drawn on the graph). The correlation between BTEX and TPH was $R^2 = 0.81$. This result shows that BTEX can generally be used to represent TPH. In a few instances concentrations were relatively low, thus may have been due to analytical variances. On the other hand, no correlation was found between BTEX and chloride concentrations (Figure 4-13). This lack of correlation may be either supporting evidence that these contaminants behave in different manners, or an indication that chloride concentrations are not affected by BTEX concentrations.

Figure 4-14 contains scatter plots comparing benzene concentrations to those of toluene, ethylbenzenes, and total xylenes. This figure shows a moderate correlation between these compounds, but there is no apparent dominance of any of the BTEX compounds.

Changes in individual BTEX compound concentrations were compared to look for evidence of preferential biodegradation. According to Wiedemeier et al. (1999), toluene and ethylbenzene may be found at lower concentrations than benzene and xylenes. However, there is no obvious chromatographic effect, where one BTEX constituent forms a much longer plume than the other constituents due to differential rates of degradation. The database was used in two ways to evaluate evidence of preferential attenuation. First, components of BTEX that had a greater number of decreasing trends might be considered more biodegradable. Secondly, the relative decrease in concentrations of BTEX components was compared to determine whether one compound degrades faster than another.

Trends classified for each BTEX component in the 159 plumes are presented in Table 4-6. These results show broad similar attenuation behaviour for each of the BTEX components, although some qualitative differences were noted.

Table 4-6. Distribution of Trends Interpreted for Individual BTEX Components (Total =159 Plumes)

Plume Trend	Benzene	Toluene	Ethylbenzene	Xylenes
Increasing	4%	3%	2%	4%
Decreasing	25%	31%	24%	24%
Stable	36%	21%	26%	32%
Unknown	22%	20%	22%	21%
Never Detected (no data)	13%	25%	26%	19%

The following assumptions were made to explain potential trends in BTEX components (Komex International Ltd., 2001b). First, because BTEX compounds were present in the original source, nondetectable concentrations measured in the most recent sampling event are interpreted as evidence of attenuation. Secondly, a decreasing trend over time implies that there is degradation of contaminants. A stable trend does not always mean that the plume is being attenuated, instead the solubility limit of a contaminant may prevent detection of increased concentrations. Finally, increasing trends imply attenuation is not occurring.

Toluene has the greatest number of decreasing trends, least number of stable trends, and second highest number of nondetects, this could be interpreted as toluene can degrade more readily than BEX compounds. Ethylbenzene was detected in fewer plumes and at lower concentrations compared to other BTEX components (Table 4-5). Ethylbenzene trends were difficult to interpret and could be attributed to ethylbenzene being a minor component of the original contaminant. Benzene had the highest number of stable plumes and the fewest nondetects. These data may reflect benzene's high solubility relative to the other BTEX compounds or indicate that it has the lowest attenuation rate, or both.

Comparisons of changes in each BTEX component relative to the other components were used to assess variations in attenuation behaviour. BTEX compounds were compared for trends using ratios of absolute and relative concentration reduction. Only plumes that were growing or shrinking were included in the data presented in Table 4-7.

Table 4-7. BTEX Compound Attenuation Ratios

Absolute Change Ratio	$\Delta B/\Delta T$	$\Delta B/\Delta E$	$\Delta B/\Delta X$	$\Delta T/\Delta E$	$\Delta T/\Delta X$	$\Delta E/\Delta X$
# Sites > 1	26	30	20	24	15	5
# Sites < 1	36	22	34	28	39	48
Sites = 1	0	0	0	0	0	1
Preferred Compound	Toluene	Neither	Xylene	Neither	Xylene	Xylene

Table 4-7 continued

Relative Change Ratio	$(\Delta B/B_o)/(\Delta T/T_o)$	$(\Delta B/B_o)/(\Delta E/E_o)$	$(\Delta B/B_o)/(\Delta X/X_o)$	$(\Delta T/T_o)/(\Delta E/E_o)$	$(\Delta T/T_o)/(\Delta X/X_o)$	$(\Delta E/E_o)/(\Delta X/X_o)$
# Sites > 1	4	7	10	9	20	14
# Sites < 1	40	29	25	19	9	14
Sites =1	16	15	18	18	20	21
Preferred Compound	Toluene	Ethyl-benzene	Xylene	Ethyl-benzene	Toluene	Neither

$\Delta B = B_{start} - B_{end}$ for a given monitoring period, same for T, E and X
$B_o = B_{start}$ for a given site, same for T, E and X

When the ratio is less than 1 (<1), the compound in the numerator has degraded less than the denominator compound, if the ratio is >1 then the numerator has attenuated more than the denominator. A ratio of <1 for the absolute change ratio indicates the numerator compound exhibited a smaller concentration decrease than the numerator (and vice versa for a ratio >1). A ratio of 1 indicates that the numerator and denominator have attenuated equally and neither appears to degrade preferentially. The numerator and denominator for the relative change ratios show the change in contaminant as a percentage of the original concentration, thus a ratio of <1 is interpreted as showing the numerator is decreasing less than the corresponding percentage change in the denominator. This was done to examine the effects of original the concentration on natural attenuation and determine whether higher original concentrations inhibit or enhance degradation.

The ratio must take into account that measured concentrations of a compound may not reflect the original source material, especially if monitoring began several years after contamination of the groundwater. In addition, BTEX concentrations in the source area are likely to vary between plumes.

The data in Table 4-7 show that benzene is never degraded preferentially. This is supported by absolute and relative change ratios. Toluene shows greater absolute changes than benzene ($\Delta B/\Delta T<1$) and greater relative changes than both benzene and xylenes. On the other hand, xylene has a greater absolute change in concentration compared to toluene and ethylbenzene. Ethylbenzene appears to have a greater relative change in concentration compared to benzene and toluene. However, ethylbenzene almost always showed a smaller absolute concentration change than xylenes, but almost the same distribution of relative changes. This suggests that there is usually less ethylbenzene originally present in the source area. This is further supported by data in Table 4-5, where the majority of ethylbenzene is between 0 and 0.99 mg/L.

4.3.7 EVALUATION OF ULTIMATE ATTENUATION ENDPOINT

Shrinking plumes were examined to assess the effectiveness of natural attenuation. The reduction in plume concentration was compared to the original concentration (at the beginning of the monitoring period) to determine how much of the BTEX contamination was attenuated over the monitoring period. Figure 4-15 shows that almost all of the BTEX concentrations in the original source area have been substantially reduced over the monitoring period. The correlation between change in BTEX concentration and original concentration gave a r^2=0.96. This means that the majority of sites with low original BTEX concentrations (<10 mg/L) were remediated to below the detection levels. It should be further noted that 28 plumes had original BTEX concentrations below 10 mg/L.

4.3.8 EVALUATION OF EXPRESSED BIODEGRADATION CAPACITY (EBC)

Figure 4-16 compares maximum BTEX concentration in each plume to the theoretical EBC calculated from TEAPs at the same monitoring well in the contaminated area. The data points are identified by the inferred overall plume trend (shrinking, stable, indeterminate or growing). When the EBC exceeds measured hydrocarbon concentrations at a site then natural attenuation is expected to be successful. The majority of plumes have low BTEX concentrations and relatively low EBC (within the bounded area). A few shrinking and stable plumes have low BTEX but high EBC. Sometimes the electron acceptors may start to increase in concentration once the contamination has been degraded.

The ratios of EBC to BTEX are summarized in Table 4-8. The data are conservative because methane concentrations were never measured so the potential EBC is missing the methane component.

Table 4-8. Summary of Expressed Biodegradation Capacity (EBC) vs. Measured BTEX Concentrations (mg/L)

Plume	EBC/BTEX < 1 (NA less likely)	EBC/BTEX > 1 (NA likely)	Not enough data to compare
Shrinking (n = 40)	7 (17.5%)	29 (72.5%)	4 (10%)
Stable (n = 74)	26 (35%)	41 (55%)	7 (10%)
Growing (n = 10)	2 (20%)	3 (30%)	5 (50%)
Indeterminate (n = 26)	8 (23%)	18 (51%)	9 (26%)

These data presented in Table 4-8 show how EBC can be used as a qualitative indicator of the likelihood of natural attenuation. However, the data should be considered in perspective, as there are cases that do not fit the growing or shrinking plume status classification based on the expectation that EBC should exceed BTEX. Furthermore, three of the plumes classified as growing have an EBC/BTEX ratio greater than 1. Closer analysis of one plume reveals there is also a high ethylene glycol concentration, and glycol may be preferentially degraded relative to BTEX. Reduced BTEX biodegradability ~~has been~~ observed for gasoline-ethanol mixtures with high ethanol concentrations (Corseuil et al., 2000).

Shrinking plumes with EBC/BTEX ratios far below 1 were examined and found to have a variety of factors that may contribute to such anomalies. Some plumes had limited historical data for calculating EBC. Others had poor background wells that may have been contaminated, thus skewing the calculated change in TEAs. Additionally, low EBC suggests that attenuation mechanisms other than biodegradation may be significant.

Table 4-9 shows the estimated relative importance of each TEAP when degrading BTEX. The data were determined by averaging the expressed biodegradation capacity for each type of electron acceptor from 159 sites (sample calculation in Section 3.2.7). Methanogenesis was not included due to the lack of methane measurements.

Table 4-9. Summary of EBC of Individual Electron Acceptors

Plume Type	O_2	NO_3^-	Fe (III)	Mn (IV)	SO_4^{2-}
Shrinking (n = 40)	1.5%	2.3%	9.9%	1.4%	84.7%
Stable (n = 74)	1.3%	1.7%	3.4%	1.2%	92.4%
Growing (n = 10)	4.3%	0.8%	9.1%	7.0%	78.9%
Indeterminate (n = 35)	1.3%	0.6%	8.8%	1.9%	87.4%

Table 4-9 clearly indicates the importance of anaerobic biodegradation mechanisms, specifically sulfate reduction (79-93%). Relatively large differences between background and plume sulfate concentrations show it as the potentially dominant electron acceptor. The data are potentially skewed because geochemistry in Western Canada can have high sulfate concentrations compared to other regions. At sites where sufficient nitrate was present, this electron acceptor was observed to have a significant contribution to intrinsic biodegradation. However, nitrate generally occurs at low concentrations, which means that this nitrate reduction has limited importance. The dominant TEAP does not appear to affect plume trend.

These results correspond well with results reported by Wiedemeier et al. (1999) for 38 downstream petroleum hydrocarbon-contaminated sites. Sulfate reduction comprised 70% of biodegradation capacity, followed by methanogenesis (16%). Denitrification (9%), aerobic respiration (3%) and iron (III) reduction (2%) contributed less than a quarter of the BTEX biodegradation mechanisms.

4.3.9 PLUME LENGTH

The estimated plume length could only be determined if there were sufficient monitoring wells at the site to delineate the plume to the point of non-detect. As a result, plume length could only be determined for 68 out of 159 PHC plumes (Figure 4-17). The plumes with multiple contaminants (non-PHC and inorganic) within or co-mingling with a BTEX plume were compared with the distribution of plume lengths for PHC and inorganics. Non-PHC plumes were not included due to limited data (n = 6); these plumes ranged between 40 and 1000 m in length. As expected, non-PHC and inorganic plumes tended to be longer than PHC plumes. This is further supported by data in Table 4-10. The median length for PHC plumes was 50 m, whereas non-PHC plumes had a median of 100 m and the median for inorganic plumes was 75 m. Caution must be taken when comparing non-PHC and inorganic plumes, because several factor such as lower detection limits and higher solubility can skew results.

Table 4-10. Distribution of Plume Length (m) for PHC, non-PHC and Inorganic Plumes

	PHC (n = 67)	Non-PHC (n = 6)	Inorganic (n= 37)
Minimum	10	40	20
10th Percentile	20	48	40
25th Percentile	37.5	60	55
Median	50	100	75
75th Percentile	100	215	200
90th Percentile	185	600	250
Maximum	775	1000	1000

Table 4-10 shows that PHC plume lengths are consistently shorter than the other plume types. The median length for PHC plumes of 50 m is similar to results reported by Newell and Connor (1998). They reported that BTEX plumes at downstream sites were shorter than other contaminant types, with BTEX plumes having a median of 65 m. Newell and Connor (1998) gave a maximum PHC plume length of 920 m compared to 775 m reported in Table 4-10.

Several non-PHC plumes contain sulfolane, a soluble gas-processing chemical with low sorptivity. This compound had a very low detection limit (0.001 mg/L) and the background concentration is usually zero. The reason that sulfolane plumes appear longer (median length = 100 m) is because they are detectable at lower concentrations, and are known to have very low biodegradability. Newell and Connor (1998) found a similar result for chlorinated solvents compared to brine releases, with median lengths of 210 m for chloride plumes, and 305 m for chlorinated ethene plumes. Chlorinated compounds were not examined in the plume-a-thon database.

Plume length versus groundwater velocity was plotted in Figure 4-18. Only 47 out of 159 sites had sufficient data to compare groundwater velocity and plume length. The correlation ($r^2 = 0.5$) suggests that plume length is partly affected by groundwater velocity. In comparison, data from 38 Air Force sites showed that plume length was reasonably correlated to retarded seepage velocity (the rate of discharge of ground water per unit area of a porous medium measured at right angle to the direction of flow) ($r^2 = 0.5$) (Wiedemeier et al., 1999). Closer inspection of 2 outlier points that appear to have a long plume length but low groundwater velocity, showed that these plumes are likely co-mingled plumes emanating from multiple sources along the flowpath, and have several separate plumes may have initially been interpreted as one long plume. Elimination of the 2 outlier points changes the r^2 to 0.6.

4.3.10 PLUME HYDROGEOLOGY

The data in this study do not show any correlation between plume characteristics and hydrogeologic conditions. However, further analysis is required to find possible relationships. The current analysis simply examined trends between 2 or 3 variables.

Table 4-11 shows the distribution of geology as it compares to plume trend (PHC plumes only). Bedrock and surficial geology are classified according to three expected ranges of permeability. Low permeability sites have either clay-rich soils or shale to mudstone bedrock, whereas high permeability sites have sand to gravel soils or sandstone bedrock. Medium permeability sites were classified as having silty soil or siltstone.

Table 4-11. Plume Trends in Bedrock and Surficial Hydrogeological Types

Plume Type	Low Permeability		Medium Permeability		High Permeability	
	S	B	S	B	S	B
Shrinking (n = 40)	5	0	12	4	9	10
Stable (n = 74)	5	2	36	2	20	9
Growing (n = 10)	2	2	4	1	1	0
Indeterminate (n = 35)	7	3	9	2	10	4

S = Soil; B = Bedrock

Many stable plumes are in medium and high permeability soil, such as silt and sand. Thus despite geological conditions that favour contaminant transport, these plumes do not appear to be growing. It is likely that several factors combine to influence attenuation behaviour including groundwater velocity, biodegradation rate, and relative concentrations of contaminants and terminal electron acceptors. Shrinking, indeterminate and growing plume trends do not appear to be affected by hydrogeologic type.

Figure 4-19 gives the distribution of estimated linear groundwater velocity in various hydrogeologic units for PHC plumes, and Table 4-12 shows the percentile distribution of groundwater velocity for each geology type. The velocities were not corrected for retardation due to insufficient data at all sites. Maximum groundwater velocities exceeded 100 m/yr at sites with highly permeable soils, or sites with both medium and high permeability geology. The low permeability geology has a narrower spread than medium and high permeability geology. Higher maximum and median velocities were found in bedrock type geology compared to surficial soil. A more detailed assessment based on measured conductivity could not be conducted due to data limitations. Most of the hydraulic conductivity data derived were from single well slug tests, however data can vary within a given hydrogeological unit for reasons unrelated to geology (Freeze and Cherry, 1979). Slug tests may not incorporate fracture influences.

Table 4-12. Distribution of Groundwater Velocity (m/yr) as a Factor of Geology Type for Petroleum Hydrocarbon Plumes

	Low permeability (Soil)	Low permeability (Bedrock)	Medium permeability (Soil)
Count	17	7	58
Minimum	0.01	0.1	0.02
10th Percentile	0.034	1.84	0.17
25th Percentile	0.3	3	0.7
Median	1	3	1.9
75th Percentile	6	11.5	5.075
90th Percentile	7.9	14.2	10.6
Maximum	12.6	16	70

Table 4-12 Continued

	Medium permeability (Bedrock)	High permeability (Soil)	High permeability (Bedrock)
Count	9	37	23
Minimum	4	0.76	0.4
10th Percentile	4	1.6	5.4
25th Percentile	10	3	8.25
Median	28.5	10	25
75th Percentile	45	25	35.5
90th Percentile	68.4	72	164.6
Maximum	142	130	250

Figure 4-20 illustrates the relationship between plume length and aquifer geology. This includes plume length for all contaminant types, PHC, non-PHC and inorganic. Table 4-13 separates the contaminant types into different categories in order to examine the effect of geology on each type of contaminant plume. The data generally show that sites with high and medium permeability soil or bedrock tend to have greater maximum plume lengths than sites with low permeability units for all contaminant types. Additionally, sites with low permeability bedrock have plumes less than 80 m long. Also, non-PHC and inorganic plumes appear to be longer than PHC plumes for all geology types.

Table 4-13. Distribution of Plume Length (m) as a Factor of Geology Type for Petroleum Hydrocarbon Plumes.

	Low permeability (Soil)			Low permeability (Bedrock)		
	PHC	**Non-PHC**	**Inorganic**	**PHC**	**Non-PHC**	**Inorganic**
Count	8	0	3	4	1	2
Minimum	15	0	55	35	40	40
10th Percentile	22	0	58	36.5	40	40
25th Percentile	28.75	0	62.5	38.75	40	40
Median	57.5	0	70	40	40	40
75th Percentile	76	0	150	47.5	40	40
90th Percentile	107.5	0	198	61	40	40
Maximum	125	0	230	70	40	40

Table 4-13 continued

	Medium permeability (Soil)			Medium Permeability (Bedrock)		
	PHC	Non-PHC	Inorganic	PHC	Non-PHC	Inorganic
Count	22	5	18	4	1	4
Minimum	15	50	25	10	1000	25
10th Percentile	25.5	54	43.5	11.5	1000	28
25th Percentile	32.5	60	50	13.75	1000	32.5
Median	50	125	60	32.5	1000	70
75th Percentile	82.5	215	113.75	137.5	1000	328.75
90th Percentile	100	386	162.5	295	1000	731.5
Maximum	375	500	500	400	1000	1000

	High permeability (Soil)			High permeability (Bedrock)		
	PHC	Non-PHC	Inorganic	PHC	non-PHC	Inorganic
Count	21	2	15	9	0	7
Minimum	15	70	20	50	0	60
10th Percentile	20	73	48	66	0	108
25th Percentile	40	77.5	60	75	0	157.5
Median	50	85	140	125	0	225
75th Percentile	90	92.5	200	500	0	225
90th Percentile	150	97	250	755	0	445
Maximum	350	1000	550	775	0	775

These results suggest that subsurface permeability may influence plume length but it does not necessarily determine plume behaviour. Other research has also shown a similar lack of correlation between plume behaviour and hydrogeology. Mace et al., (1997) found that hydrogeologic site characteristics did not explain the variation in average plume length or contaminant concentrations. The aquifer system, aquifer texture, hydraulic conductivity and the presence of free product did not have much influence on the median plume length. Rice et al., (1995) concluded that correlations between a hydrogeological variable and plume length showed no indications of interaction. The lack of correlation in the data was attributed to the presence of other controlling variables such as source mass and biodegradation rate. Scatter in the hydrogeological data, cyclic changes in hydrogeological variables and general site complexity also were factors that can explain the absence of any correlation.

4.3.11 BIODEGRADATION RATES

Biodegradation reactions of organics in groundwater occur at rates that are a function of the prevailing environmental conditions, such as temperature and availability of electron acceptors and donors (Wiedemeier et al., 1999). Various kinetic models can be used to represent the biodegradation of organic chemicals. First order kinetics are often used to model biological processes, including biodegradation of organic contamination in groundwater. First order kinetics can be expressed by:

$$C_t = C_O e^{-kt} \qquad [21]$$

where, C_t = biodegraded concentration of the chemical

C_O = starting concentration

k = rate of decrease of the chemical

t = time of monitoring chemical

e = base of the natural log = 2.7183

Rearrangement of this equation gives:

$$\ln(C_t / C_O) = -kt \qquad [22]$$

Plotting ln (C_t/C_O) versus time will give a slope that is the maximum rate constant, k. Comparing rate constants can be easier if they are converted to a half-life ($t_{1/2}$), which is the time it takes to biodegrade half the contaminant. This can be calculated by:

$$t_{1/2} = \frac{\ln(0.5)}{-k} = \frac{0.693}{k} \qquad [23]$$

Biodegradation rates were calculated assuming that first order kinetics describes the process. Rates were derived from the slope of a line fitted to a plot of concentration on a log scale vs time. Data to estimate biodegradation rates were only available at 26 PHC plumes (summarized in Table 4-14). The distribution of biodegradation rates for total BTEX is illustrated in Figure 4-21. For comparison, this figure also shows rate data from Rifai et al (1995) derived from studies in the United States.

Table 4-14. Distribution of First Order Rate Constants (day^{-1}) for the Components of BTEX and total BTEX from Rifai et al. (1995) and the CORONA database (% of Total Sites)

Literature	**0.0001-0.00049**	**0.0005-0.0009**	**0.001-0.0049**	**0.005-0.009**	**>0.01**
Benzene	10	10	40	40	0
Toluene	12.5	0	50	12.5	25
Ethyl-benzene	0	0	71.4	14.3	14.3
Xylene	0	0	55.6	22.2	22.2
Total BTEX	0	7.7	46.2	30.8	15.4
CORONA	**0.0001-0.00049**	**0.0005-0.0009**	**0.001-0.0049**	**0.005-0.009**	**>0.01**
Benzene	9.1	40.9	36.4	13.6	0
Toluene	12	12	64	8	4
Ethyl-benzene	16	24	48	4	8
Xylene	9.1	36.4	40.9	13.6	0
Total BTEX	3.8	19.2	65.4	7.7	3.8

First order biodegradation rate constants derived from the plume-a-thon database ranged from 0.00033 to 0.017 day^{-1}, with the majority of plumes lie between 0.001 and 0.005 day^{-1}. The mean rates of each component of BTEX and total BTEX were very similar (0.00211 to 0.00292). Using median values, plume half-lives for the individual BTEX components and total BTEX were 1.9, 0.9, 0.9, 1.5 and 1.1 years respectively. In contrast, Suarez and Rifai (1999) found that the overall first-order rate constants for BTEX ranged from 1 to 0.483 day^{-1}, which is a wider spread than the results in this study. Also, mean values for BTEX components were higher: 0.06, 0.25, 0.12 and 0.04 day^{-1} for BTEX components respectively. Typical rate constants for anaerobic biodegradation compiled for a number of studies in Wiedemeier et al. (1999) range from <0.001day^{-1} up to 0.06 day^{-1} (half-lives range from >3 years to 12 days), depending on the BTEX component. BTEX rate constants appear to range over 3 orders of magnitude and often benzene degradation is not observed.

Rates in the literature appear higher than the biodegradation rates in this study, which may have several causes. The data obtained by Suarez and Rifai (1999) were from 230 studies located in the United States, where warmer temperatures may result in higher degradation rates. Furthermore, the literature data concentrates on downstream sites, whereas the CORONA database contains mainly upstream oil and gas sites that often have co-contaminants that may affect biodegradation rates. Lastly, some of the CORONA sites have limited data sets that may influence interpretation of overall plume attenuation.

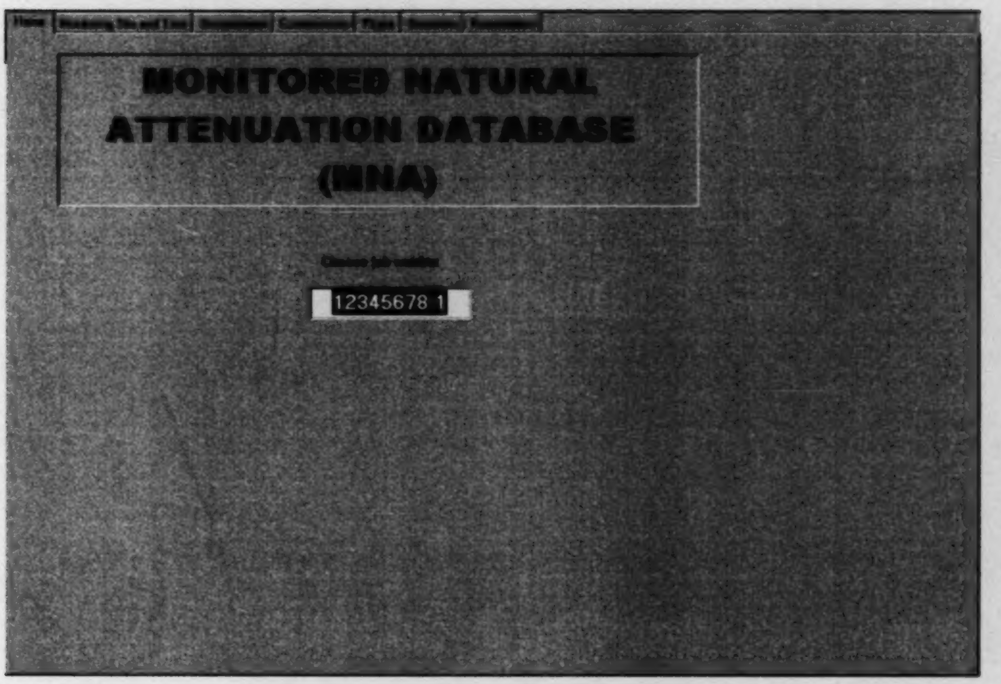
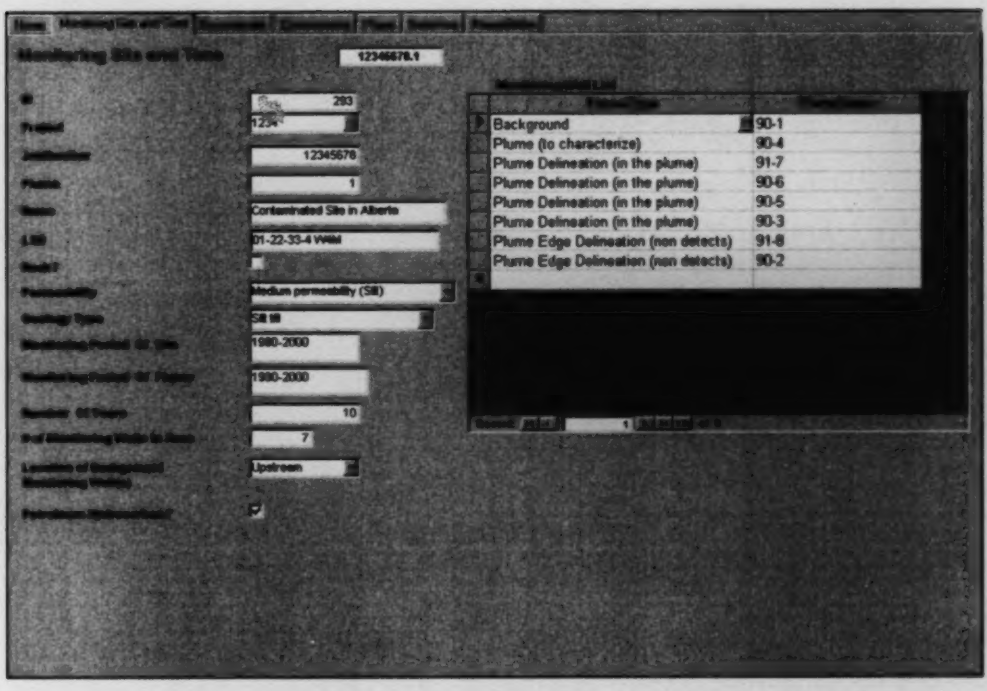

Home
MONITORED NATURAL ATTENUATION DATABASE (MNA)
12345678 1

Figure 4-1. Home page for NA database



12345678.1

293

1234

12345678

1

Contaminated Site in Alberta

01-22-33-4 W4M

☐

Medium permeability (Silt)

Silt 18

1980-2000

1980-2000

10

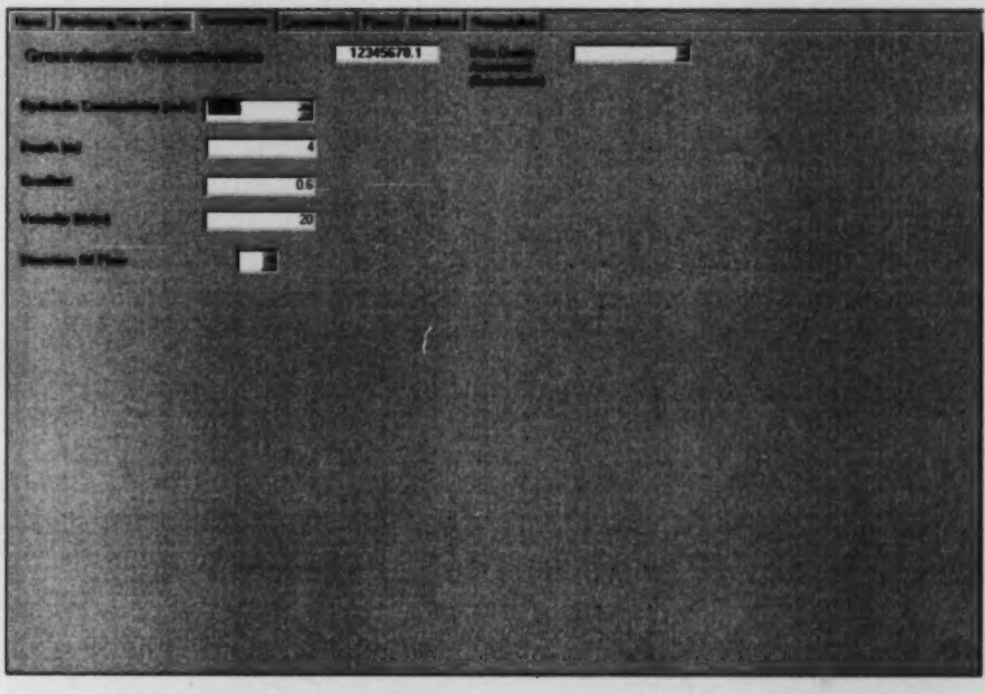
7

Upstream

☑

Background	90-1
Plume (to characterize)	90-4
Plume Delineation (in the plume)	91-7
Plume Delineation (in the plume)	90-6
Plume Delineation (in the plume)	90-5
Plume Delineation (in the plume)	90-3
Plume Edge Delineation (non detects)	91-8
Plume Edge Delineation (non detects)	90-2

Figure 4-2. Page for Monitoring Site and Time Data in the NA Database Form



Groundwater Characteristics

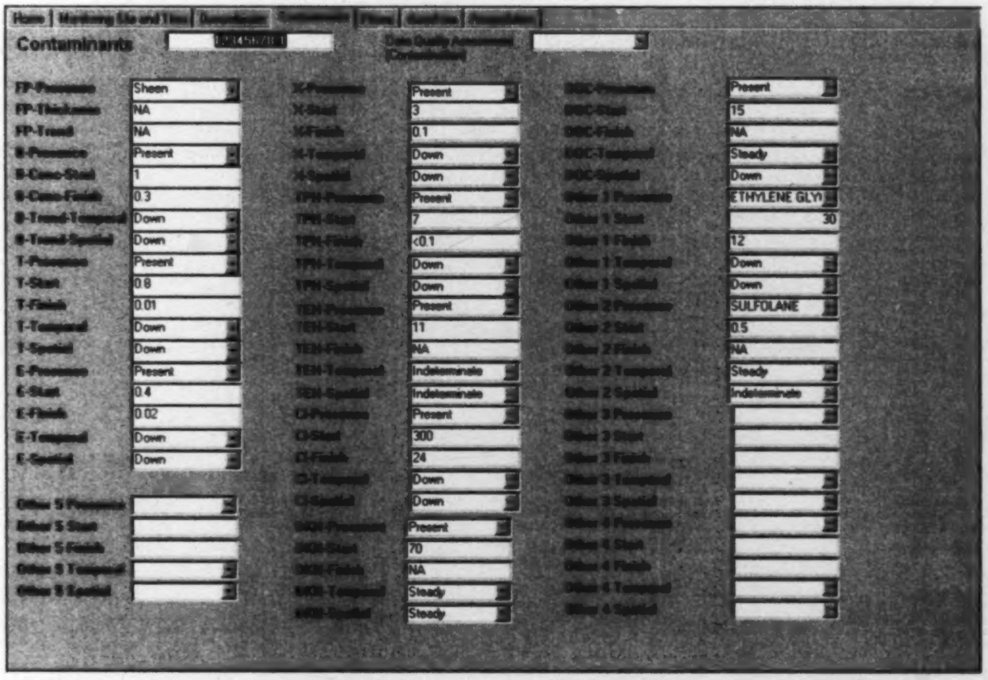
12345670.1

4

0.6

20

Figure 4-3. Page for Groundwater Characteristics in the NA Database Form

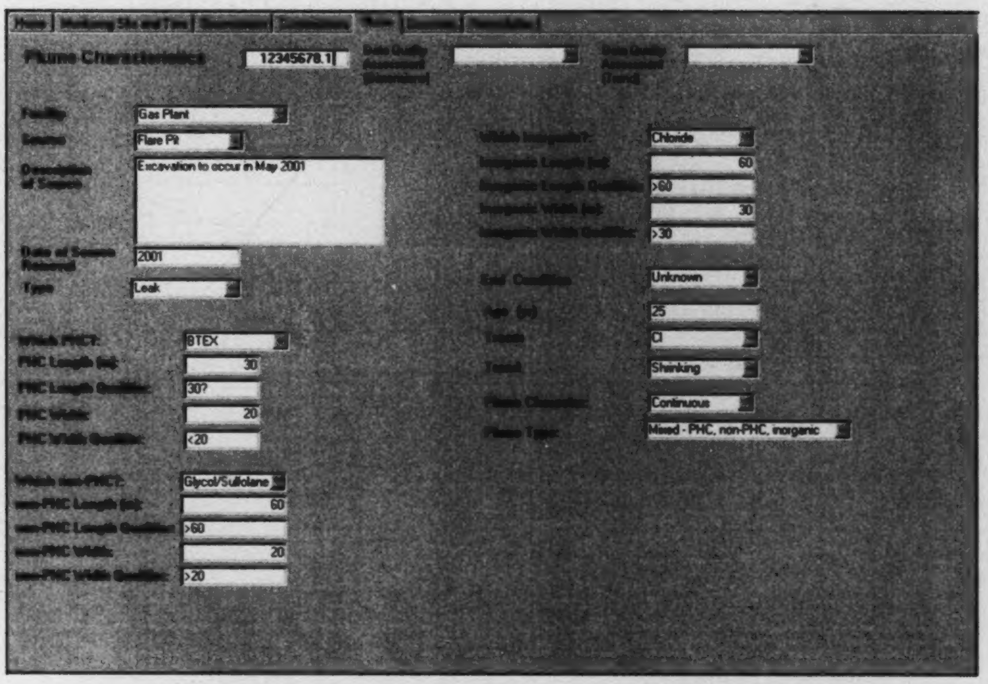


Contaminants 12345678 1

Data Quality Assessment (Contamination)

FP-Presence	Sheen	X-Presence	Present	DOC-Presence	Present
FP-Thickness	NA	X-Start	3	DOC-Start	15
FP-Trend	NA	X-Finish	0.1	DOC-Finish	NA
B-Presence	Present	X-Temporal	Down	DOC-Temporal	Steady
B-Conc-Start	1	X-Spatial	Down	DOC-Spatial	Down
B-Conc-Finish	0.3	TPH-Presence	Present	Other 1 Presence	ETHYLENE GLY
B-Trend-Temporal	Down	TPH-Start	7	Other 1 Start	30
B-Trend-Spatial	Down	TPH-Finish	<0.1	Other 1 Finish	12
T-Presence	Present	TPH-Temporal	Down	Other 1 Temporal	Down
T-Start	0.8	TPH-Spatial	Down	Other 1 Spatial	Down
T-Finish	0.01	TEH-Presence	Present	Other 2 Presence	SULFOLANE
T-Temporal	Down	TEH-Start	11	Other 2 Start	0.5
T-Spatial	Down	TEH-Finish	NA	Other 2 Finish	NA
E-Presence	Present	TEH-Temporal	Indeterminate	Other 2 Temporal	Steady
E-Start	0.4	TEH-Spatial	Indeterminate	Other 2 Spatial	Indeterminate
E-Finish	0.02	Cl-Presence	Present	Other 3 Presence	
E-Temporal	Down	Cl-Start	300	Other 3 Start	
E-Spatial	Down	Cl-Finish	24	Other 3 Finish	
		Cl-Temporal	Down	Other 3 Temporal	
Other 5 Presence		Cl-Spatial	Down	Other 3 Spatial	
Other 5 Start		[illegible]-Presence	Present	Other 4 Presence	
Other 5 Finish		[illegible]-Start	70	Other 4 Start	
Other 5 Temporal		[illegible]-Finish	NA	Other 4 Finish	
Other 5 Spatial		[illegible]-Temporal	Steady	Other 4 Temporal	
		[illegible]-Spatial	Steady	Other 4 Spatial	

Figure 4-4. Page for Contaminant Concentrations (mg/L) in the NA Database Form



Plume Characteristics 12345678.1

Data Quality Assessment (Dimensions):

Data Quality Assessment (Trend):

Facility: Gas Plant

Source: Flare Pit

Description of Source: Excavation to occur in May 2001

Date of Source Removal: 2001

Type: Leak

Which PHC?: BTEX

PHC Length (m): 30

PHC Length Qualifier: 30?

PHC Width: 20

PHC Width Qualifier: <20

Which non-PHC?: Glycol/Sulfolane

non-PHC Length (m): 60

non-PHC Length Qualifier: >60

non-PHC Width: 20

non-PHC Width Qualifier: >20

Which Inorganic?: Chloride

Inorganic Length (m): 60

Inorganic Length Qualifier: >60

Inorganic Width (m): 30

Inorganic Width Qualifier: >30

End Condition: Unknown

Age (yr): 25

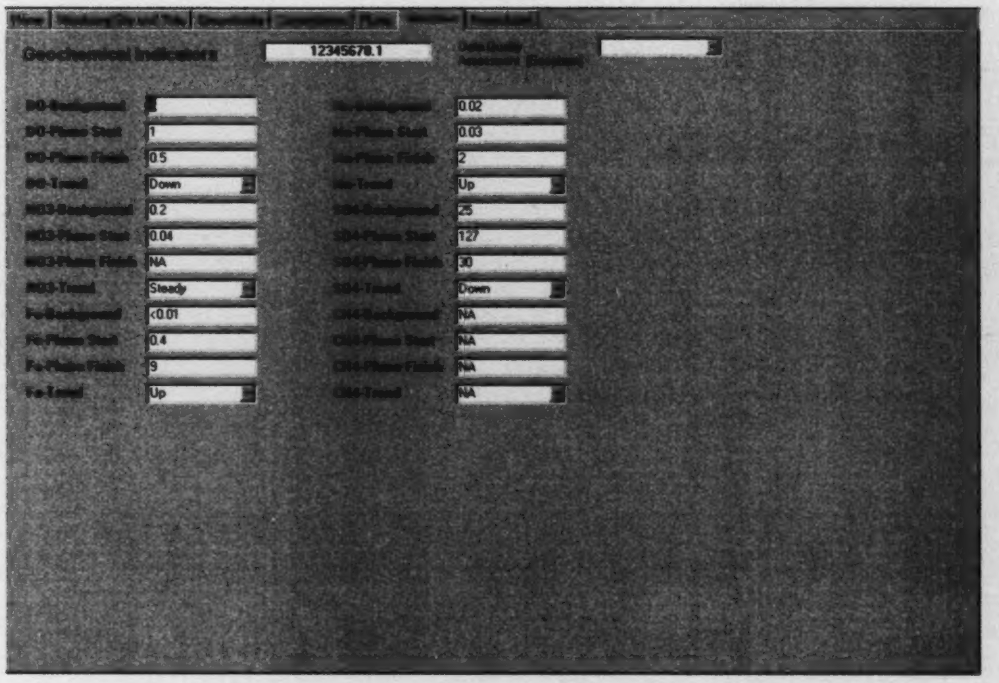
Tracer: Cl

Trend: Shrinking

Plume Character: Continuous

Plume Type: Mixed - PHC, non-PHC, inorganic

Figure 4-5. Page for Plume Characteristics in the NA Database Form

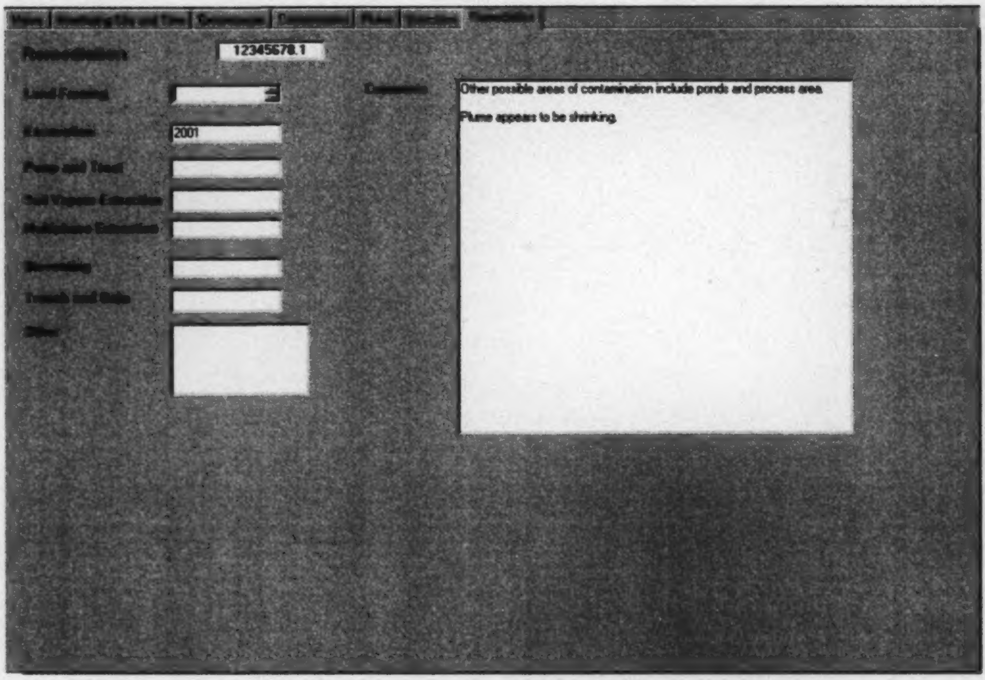


Geochemical Indicators: 12345678.1

Data Quality Assessment: [illegible]

DO-Background	[illegible]	Mn-Background	0.02
DO-Phase Start	1	Mn-Phase Start	0.03
DO-Phase Finish	0.5	Mn-Phase Finish	2
DO-Trend	Down	Mn-Trend	Up
NO3-Background	0.2	SO4-Background	25
NO3-Phase Start	0.04	SO4-Phase Start	127
NO3-Phase Finish	NA	SO4-Phase Finish	30
NO3-Trend	Steady	SO4-Trend	Down
Fe-Background	<0.01	CH4-Background	NA
Fe-Phase Start	0.4	CH4-Phase Start	NA
Fe-Phase Finish	9	CH4-Phase Finish	NA
Fe-Trend	Up	CH4-Trend	NA

Figure 4-6. Page for Geochemical Indicators in the NA Database Form



[illegible] 12345678.1

Land Farming

[illegible] 2001

Pump and Treat

Soil Vapor Extraction

Multiphase Extraction

[illegible]

Trench and Gate

Other

Comments: Other possible areas of contamination include ponds and process area.

Plume appears to be shrinking.

Figure 4-7. Page for Remediation in the NA Database Form

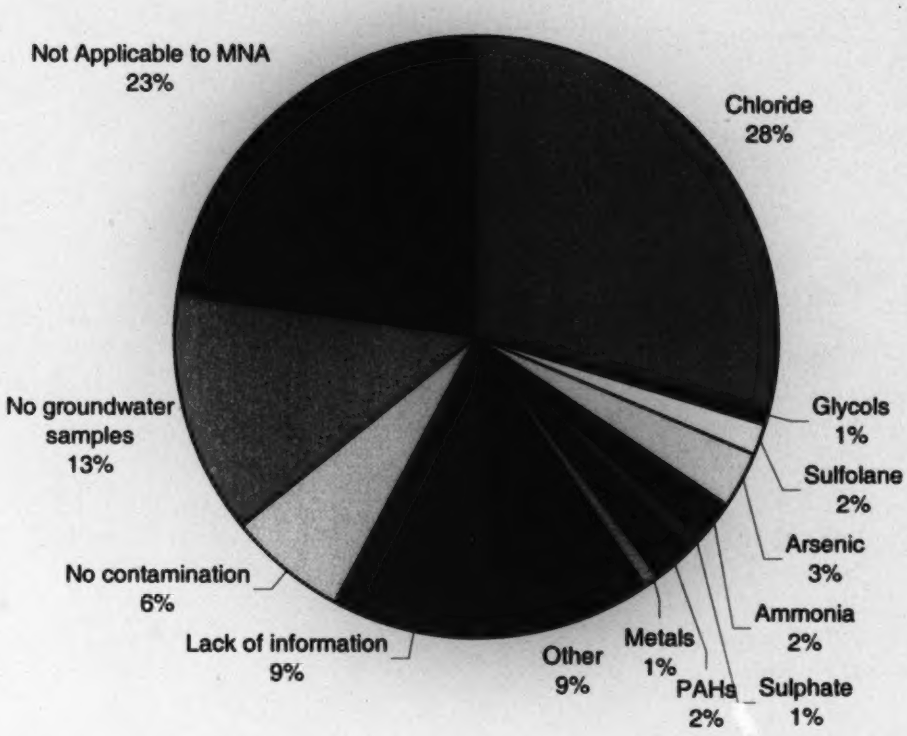


Figure 4-8. Distribution of Uncharacterized Sites (Total = 128)

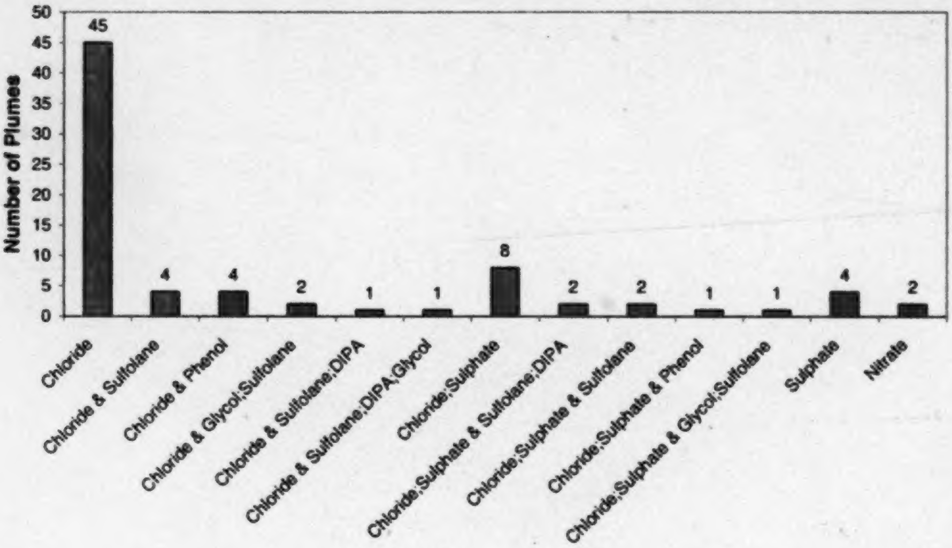
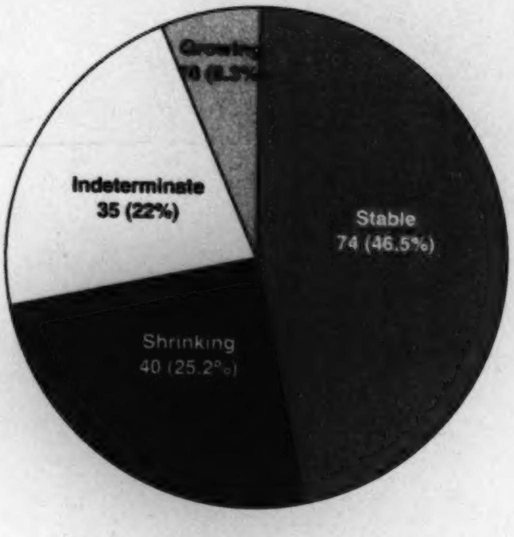
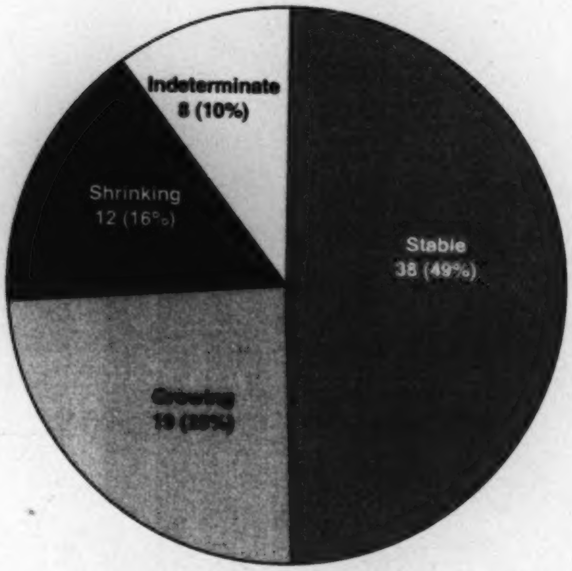


Figure 4-9. Distribution of Non-Petroleum Hydrocarbon Plume Types (Total = 77 Plumes)



Petroleum Hydrocarbon Plume Trends (Total = 159)



Non Hydrocarbon Plume Trends (Total = 77)

Figure 4-10. Petroleum Hydrocarbon and Non-Petroleum Hydrocarbon Plume Trends (Total = 236)

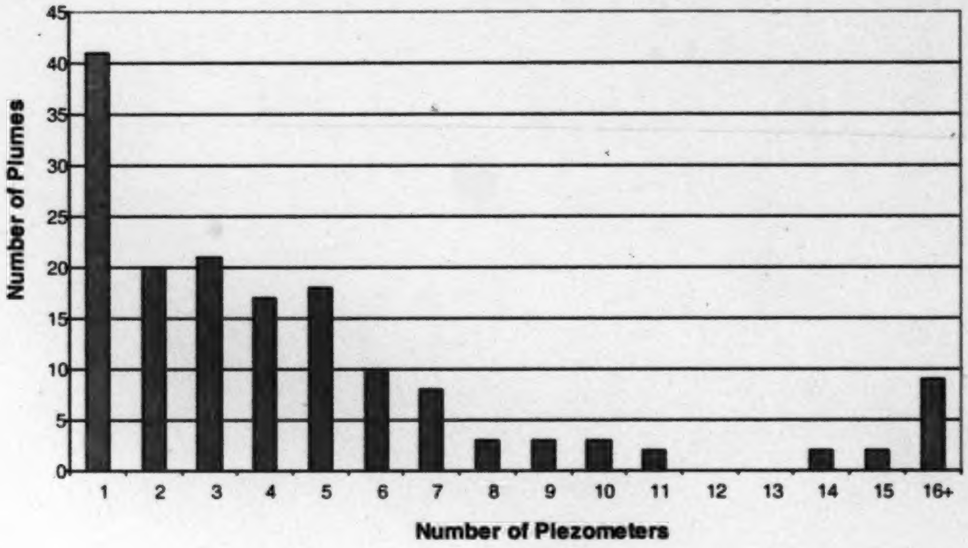


Figure 4-11. Number of Monitoring Wells used to Characterize each Petroleum Hydrocarbon Plume (Total = 159)

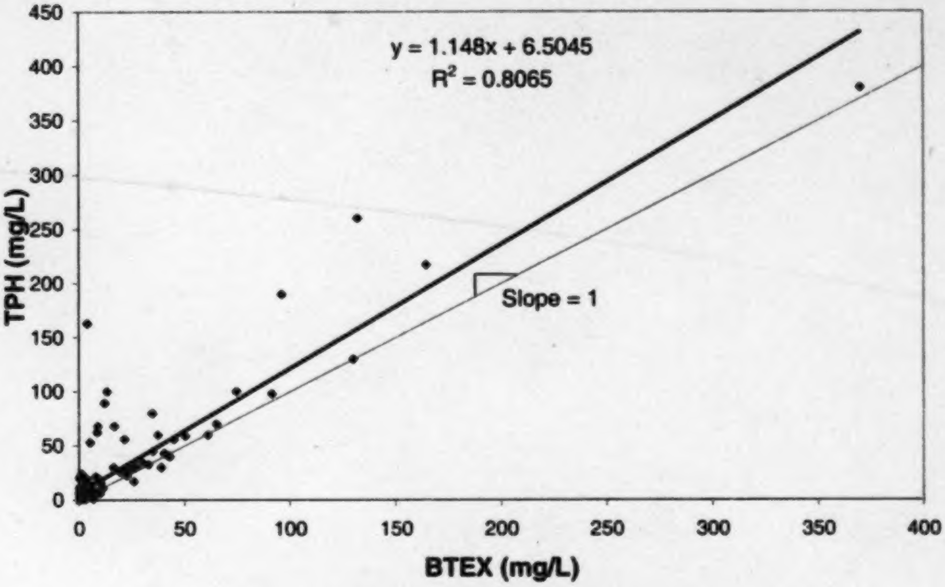


Figure 4-12. TPH vs. BTEX (Total Plumes = 141)

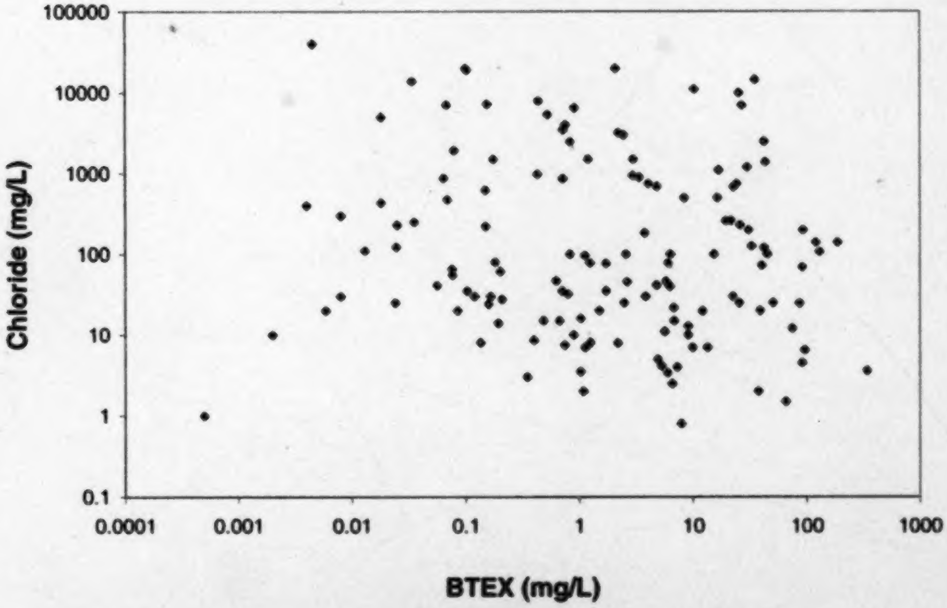
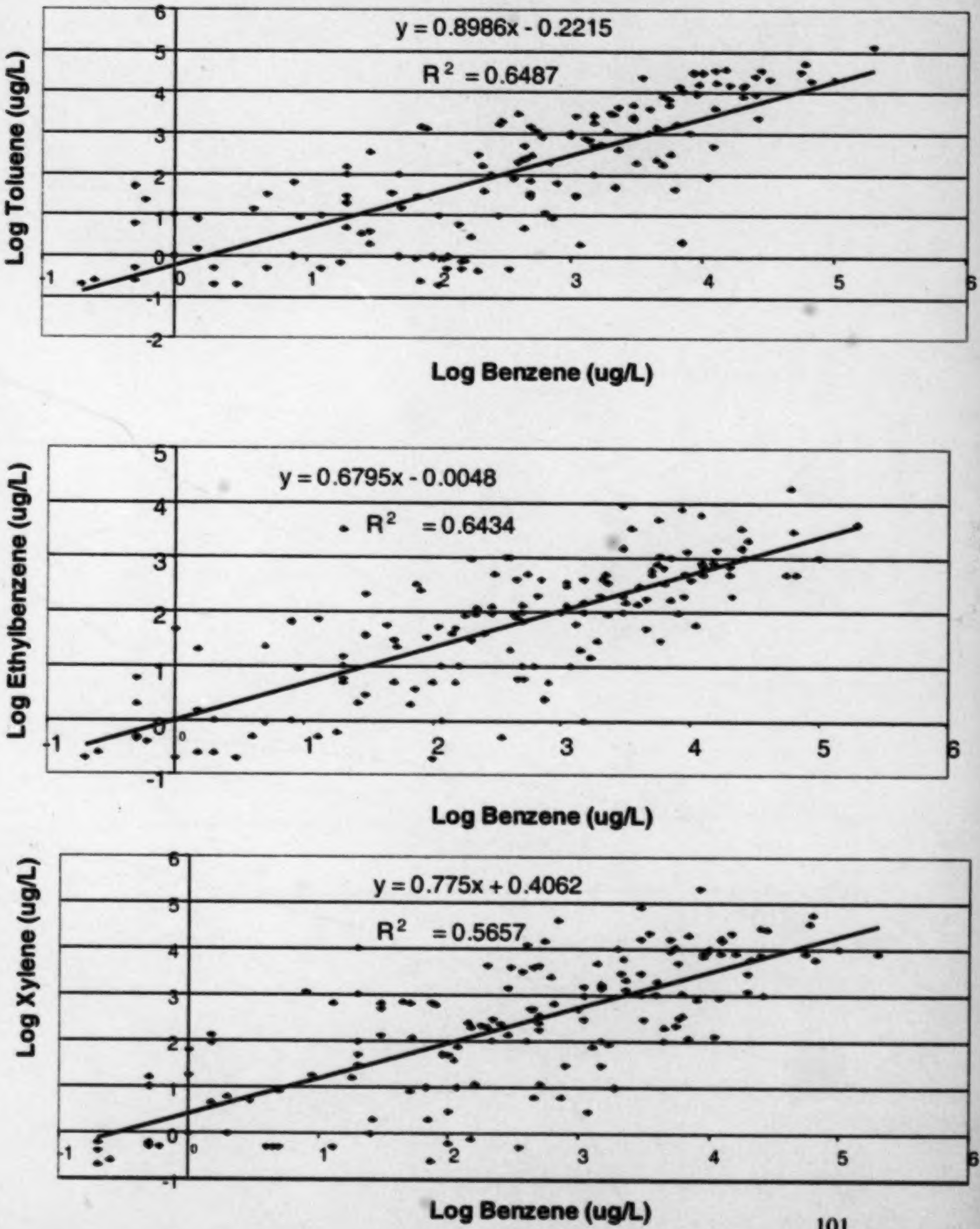


Figure 4-13. Chloride Concentration vs. BTEX Concentration for Petroleum Hydrocarbon Plumes (Total Plumes = 137)

Figure 4-14. Concentrations of Toluene, Ethylbenzene and Xylenes vs. Benzene



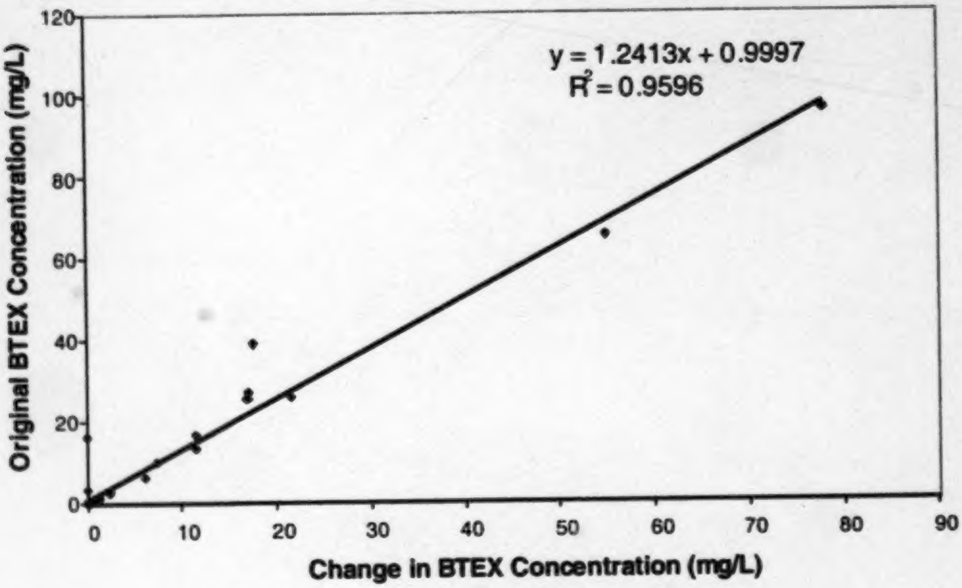


Figure 4-15. BTEX vs. Change in BTEX Concentration for Shrinking Plumes (Total Plumes = 40)

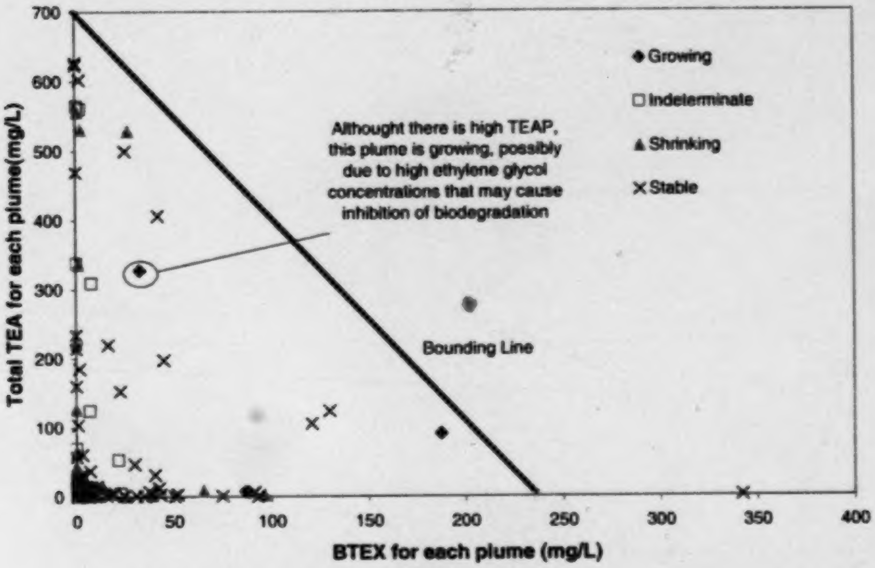


Figure 4-16. Terminal Electron Acceptor Concentration vs. Total BTEX for Growing, Indeterminate and Stable Plumes (Total Plumes = 159)

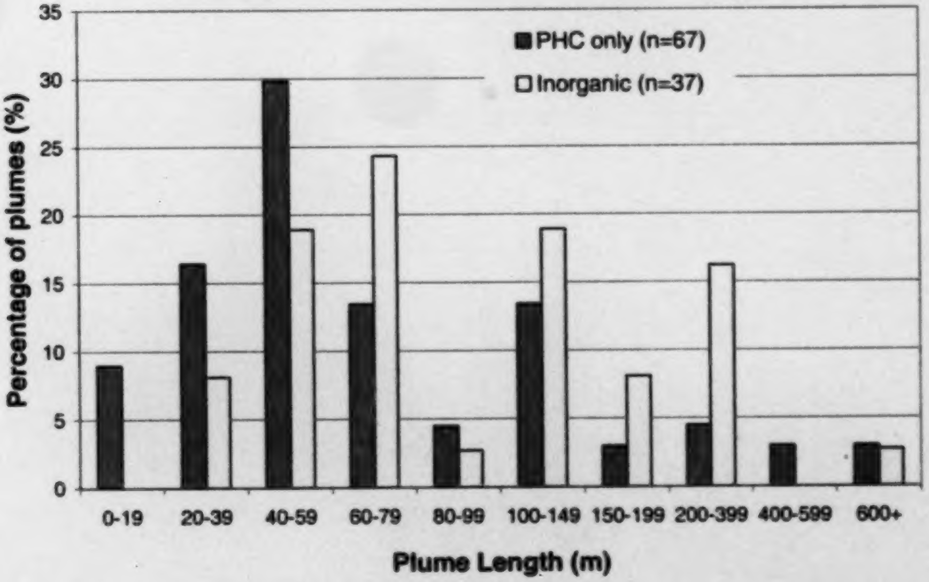


Figure 4-17. Plume Length Distribution for PHC and Inorganic Plumes

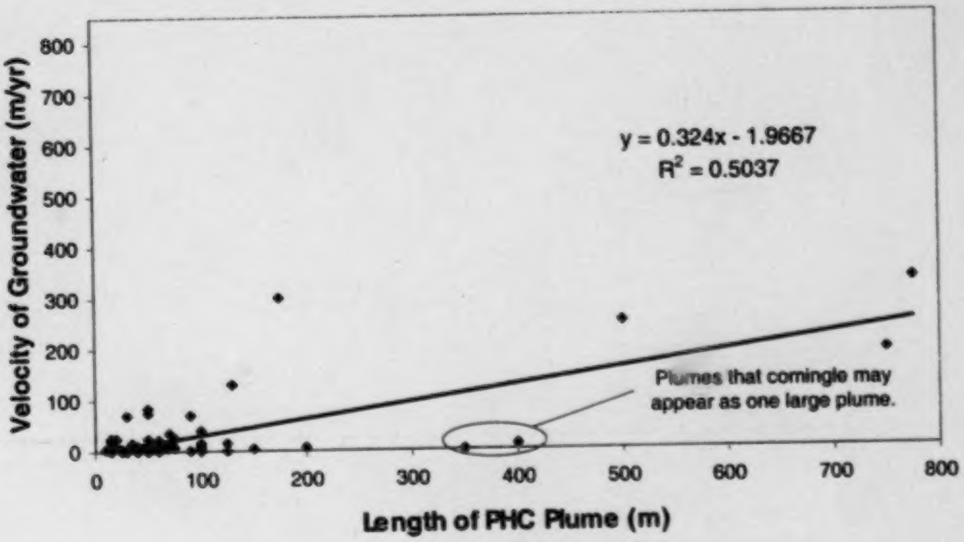
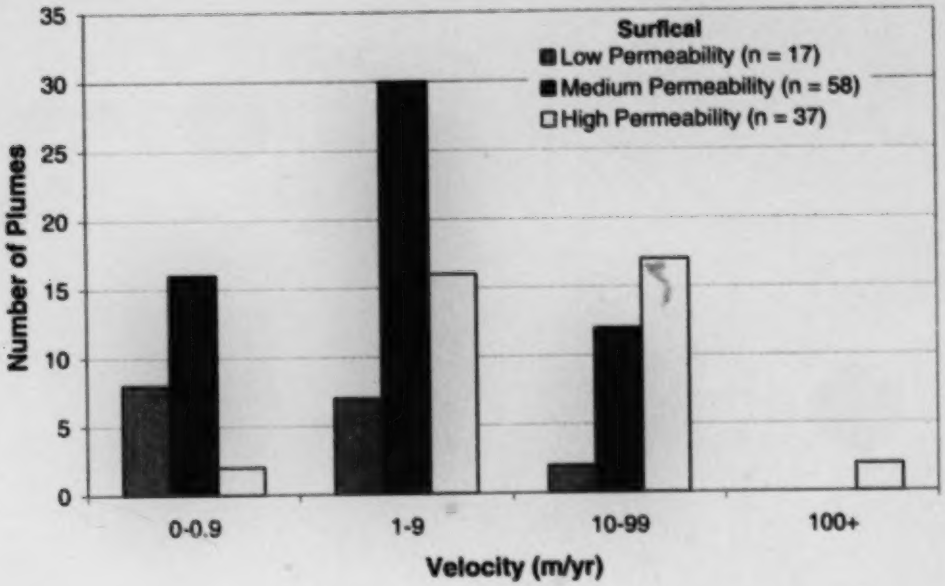


Figure 4-18. Groundwater Velocity vs. Plume Length (Total Plumes = 47)



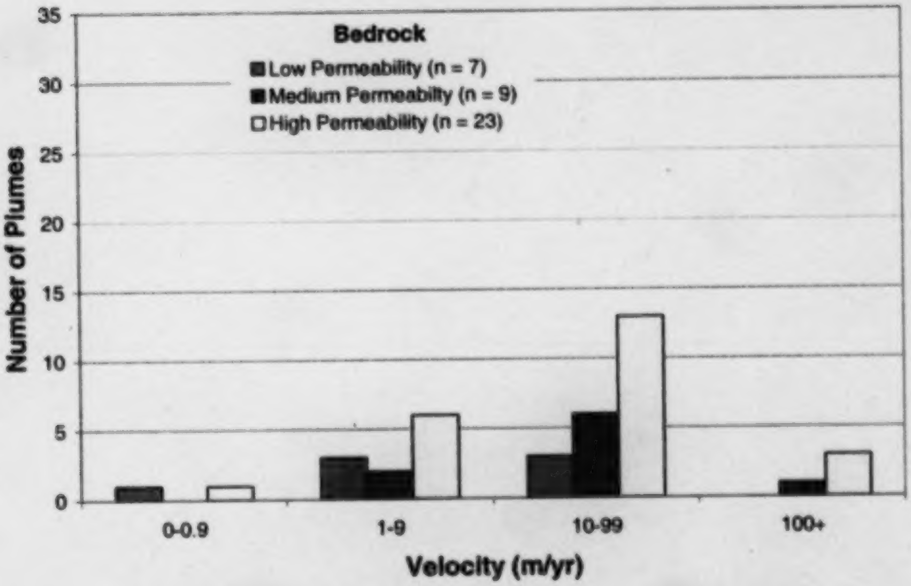


Figure 4-19. Distribution of Groundwater Velocity (m/yr) in Various Hydrogeologic Units

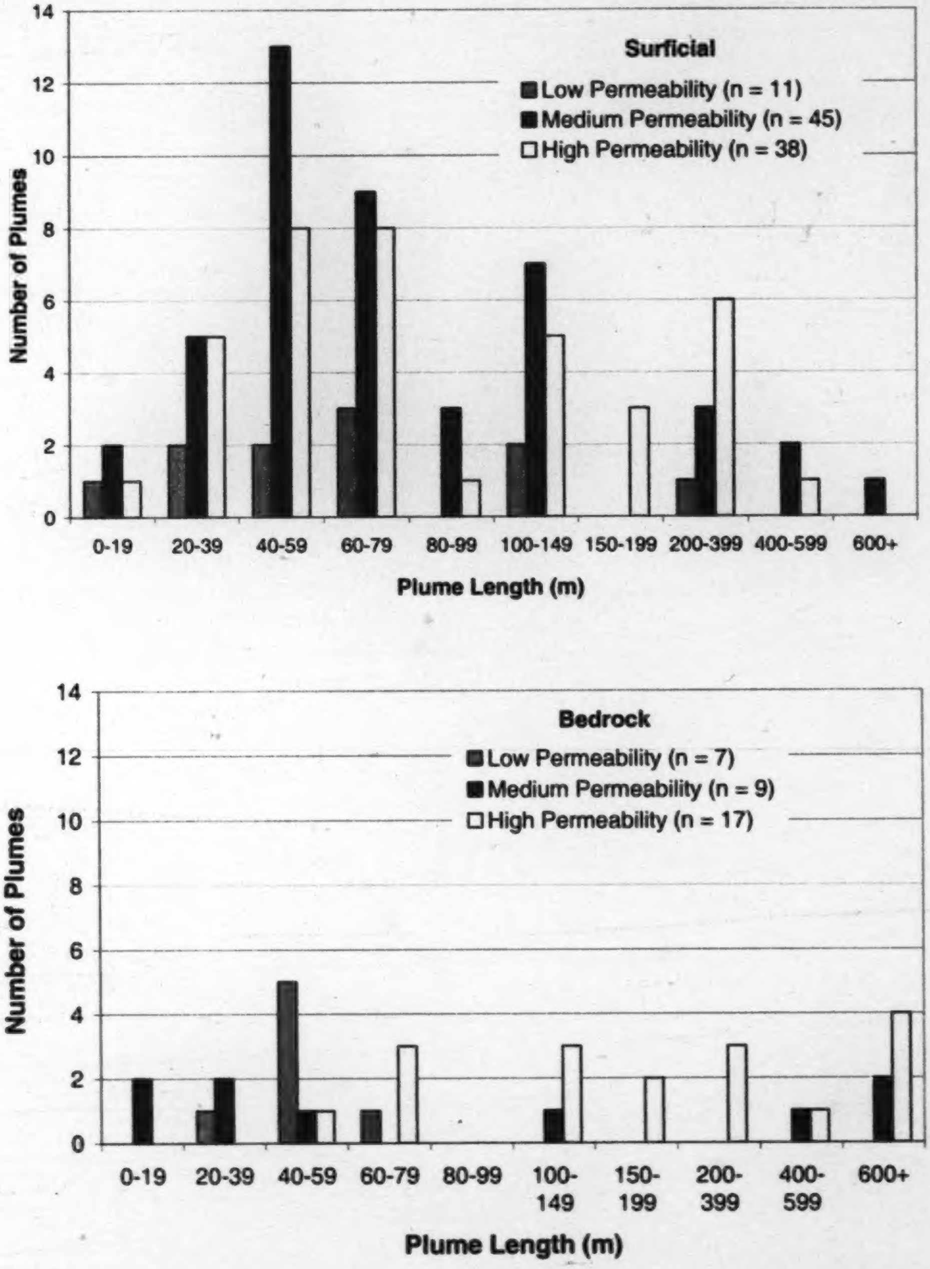


Figure 4-20. Distribution of Plume Length (m) in Various Hydrogeologic Units

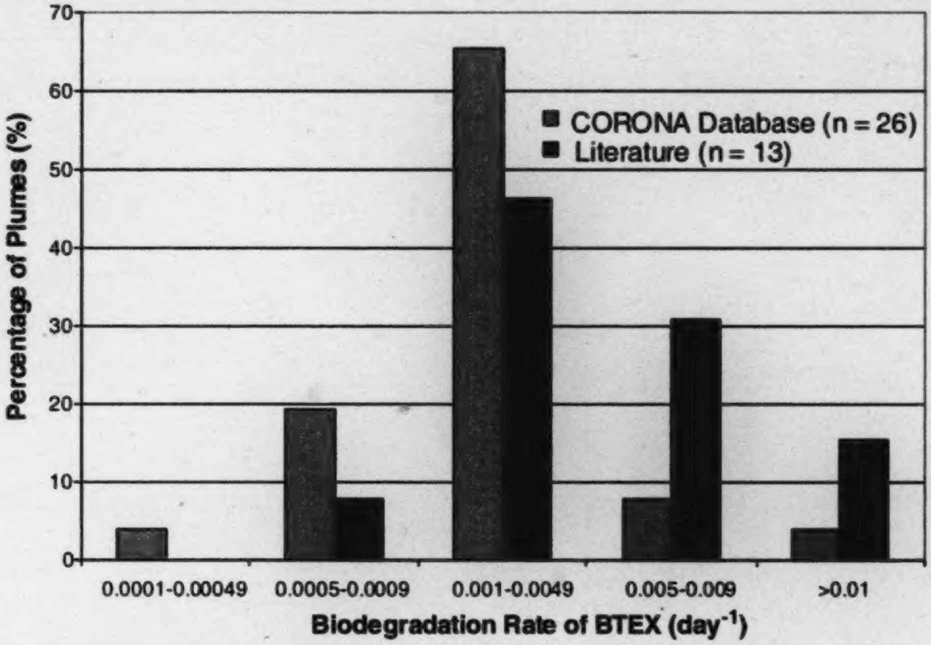


Figure 4-21. Petroleum Hydrocarbon First Order Biodegradation Rate Constants

5. CASE STUDY - SITE INFORMATION AND METHODOLOGY

5.1 INTRODUCTION

Previous investigations by Komex at the study site indicate that NA may be viable, but more evidence is necessary to confirm that NA is effective. Existing evidence includes decreasing contaminant concentrations over time at various locations across the site, and measurements of geochemical indicators along the plume consisting of depleted terminal electron acceptors and increases in microbial metabolic by-products. An additional factor in this study is that the compounds of interest are the TEH range of hydrocarbons (C_{11}-C_{30}) rather than BTEX, since BTEX concentrations are very low in the groundwater. Further evaluation of NA was undertaken by conducting microbial enumeration and activity experiments that looked for evidence of various microbial communities and their ability to biodegrade diesel.

5.2 SITE INFORMATION

The regional geology consists of sandstone, siltstone and mudstone of the Paskapoo Formation (Komex International Ltd., 1999). This formation is an important source of water for the surrounding area (Komex International Ltd., 2001a). The sandstone unit is continuous with occasional mudstone units (less than 1.0 m thick) and there appear to be no other significant geological strata (Komex International Ltd., 2001a). A thin surficial sand and gravel unit or clay till overlies bedrock. The monitoring well screens are in highly fractured fine to medium grained sandstone units. The groundwater table is more than 30 m below the surface (Komex International Ltd., 1999).

North Prairie Creek runs near the site, at the base of the hill (Figure 2-2). The groundwater table is below the base of the creek; thus the creek recharges the groundwater table. Groundwater flow is generally towards to the northeast and north, away from the creek. The rock fracture geometry and distribution likely results in variation in groundwater flow patterns. The maximum groundwater velocity at this site rarely exceeds 250 m/yr.

The study site has a total of 32 monitoring wells extending into the water table (Figure 2-2). In addition, 7 residential drinking water wells are also sampled on a regular basis to ensure there is no contamination. Apart from wells with free-product, the majority of monitoring wells have a dedicated Waterra pump and tubing that are used to sample groundwater.

Regular analysis was conducted for BTEX, TPH, TEH, metals and routine potability (alkalinity, bicarbonate, calcium, carbonate, chloride, conductivity, hardness, hydroxide, ion balance, iron, manganese, nitrate, nitrate plus nitrite, nitrite, pH, potassium, sodium, total dissolved solids, and sulfate). Appendix A contains the results of routine analysis since regular monitoring started in 1996.

5.3 FIELD SAMPLING FOR HYDROCARBON CONTAMINATION

Groundwater sampling at the study site associated with this study occurred on three separate occasions. Routine water samples from all wells were collected the first time with equipment and personnel from Komex International on September 21st and 22nd, 2000. Duplicate samples were taken from wells 3 and 8. Additionally, samples for metal and phosphate analysis were field filtered and acidified immediately after sampling.

The second sampling event occurred on October 23rd, 2000 using a bladder pump system from the University of Alberta. Groundwater samples were taken within the plume (well 3), upstream (well 16) and downstream (well 8) for microbial enumeration and anaerobic laboratory microcosm experiments. Free-phase product was extracted from the monitoring well within the plume.

The third sampling event occurred on May 22nd, 2001 using equipment from the University of Alberta. Groundwater samples were taken near the source of the plume (well 3) for aerobic laboratory microcosm microbial experiments. Samples were also taken at various other wells (3, 4, 7, 8, 10, 16, and 19) for methane analysis.

An appropriate groundwater sampling method was selected using a number of criteria, including the type of analyses to be conducted on the sample, the type of sample to be collected (grab, composite, or integrated) and the sampling depth (Byrnes, 1994).

Groundwater sampling at the study site used various sampling methods depending on the purpose of the samples. For routine chemical analysis, a Waterra pump was used. Groundwater for the microcosm was collected using a bladder pump to minimize stripping of VOCs.

5.3.1 GROUNDWATER SAMPLING – INITIAL CHEMICAL ANALYSIS

Collected groundwater samples were analyzed by Maxxam Analytics, Inc. for the presence of hydrocarbons (includes all components of BTEX, TPH and TEH), terminal electron acceptors (nitrate and sulfate), biodegradation by-products (manganese (II) and iron (II)) and a variety of other parameters. The complete chemical analysis can be found in Appendix B. Electrical conductivity, redox potential, pH, temperature and dissolved oxygen were measured on site at each well. Dissolved oxygen and temperature were measured using a probe that was lowered into the well, whereas pH, redox potential and electrical conductivity were measured from samples that were pumped to the surface. Monitoring wells that contained free-product were not tested for these parameters. Water level and interface thickness was measured with an interface probe. All probes were rinsed thoroughly with distilled water between each measurement and with methanol if any free-product was present.

For the first sampling event, a Waterra pump was used to sample the monitoring wells. The majority of monitoring wells had dedicated Waterra pumps. Wells were purged for approximately 10 minutes to ensure that water from the bedrock formation was being sampled, not the standing water in the well. All samples were extracted carefully to prevent the loss of volatiles. The Waterra pump was turned off and the water was gently poured down the side of the sample vials. Monitoring wells 3 and 8 were additionally sampled for nutrients including ammonia and phosphate. Once the dissolved metals and nutrient samples were pumped to the surface they were subsequently field filtered using a peristaltic pump. Extra samples were also taken for a semi-volatile hydrocarbon scan. Table 5-1 lists the various containers and preservatives that were used to store the samples until analysis. All samples were stored in coolers overnight before being transported to Maxxam Analytics for analysis.

A new Waterra pump was installed at monitoring well 3, since this well previously did not have a dedicated pump due to the presence of free-product. A bailer was used to remove as much free-product as possible. The interface probe was used to determine if the free-product had been completely removed. This was necessary because free-phase hydrocarbons could potentially sorb to the Waterra tubing and contaminate the pump.

Table 5-1. Groundwater Sampling Summary

Analyte	Container Volume and Type	Preservative
BTEX and TPH	Two 40 mL glass vials	None
TEH	Two 1-L amber glass	None
Routine Potability	Two 500 mL plastic bottles	None
Dissolved Metals	Two 250 mL plastic bottles	1.25 mL 1:1 HNO_3
Nutrients	Two 250 mL plastic bottles	2.5 mL 12.5% H_2SO_4
Semi-volatile open scan	1-L amber glass	None

The Waterra sampling method is relatively rapid and inexpensive to perform. However, the pumping action may introduce oxygen, release volatiles and stir up the groundwater in the formation. If there is free product in the well there is the possibility of it contaminating the groundwater sample during pumping, which may result in elevated contaminant concentrations.

5.3.2 GROUNDWATER SAMPLING FOR MICROBIAL ANALYSIS

5.3.2.1 MPNs and Anaerobic Microcosm Experiments

Groundwater samples were obtained from monitoring wells upstream, within the plume and downstream. Groundwater was extracted for microbial enumeration at all wells and for microcosms at well 3.

A Well Wizard pneumatic bladder pump was used to sample monitoring wells 3 and 8. Water enters the pump on an alternating fill and discharge cycle. The bottom check ball is pushed upwards during the fill cycle, allowing water to enter the pump, while the upper check ball prevents any water in the discharge line from escaping back into the pump. Compressed air then squeezes the bladder which forces the water into the discharge line, and the bottom check ball stops water entering the pump until the next fill cycle (Byrnes, 1994). The use of positive displacement and a low flow rate by the bladder pump minimizes the loss of volatiles contaminants or aeration and oxidation of geochemical constituents (Johns, 1999).

The water level and hydrocarbon free-phase interface were measured at all monitoring wells prior to pumping. Only well 3 contained free-product, which was bailed from the well and collected in a 4-L glass jug. Once the hydrocarbon was removed, the bladder pump was lowered rapidly into the well to minimize contact with any remaining free-product. The wells were purged for 10 minutes to remove standing water and ensure that formation water was being sampled. During purging the refill and discharge time were optimized for an efficient pumping rate. Table 5-2 summarizes the pumping information for wells 3 and 8. The recharge rate of groundwater from the formation was rapid, thus drawdown was not a concern. Nonetheless, the water level was measured periodically during and after purging.

Once the wells were purged, water was slowly pumped into a flow through cell containing probes that measured pH, redox, electrical conductivity and temperature. These parameters were measured continuously until they stabilized. A sample of water was tested for DO using a Chemets Kit, which uses colourmetric analysis to determine the concentration of DO. Water was then gently poured down the side of the 4-L sample vials so as not to introduce oxygen and prevent the loss of volatiles. The bottles were filled to overflowing and then capped so excess air would not enter the sample. Only one 4-L bottle was filled at monitoring well 8, and twenty-one bottles were filled at well 3. Following sampling, the final water level and interface measurements as well as parameters from the flow-through cell were collected.

Prior to sampling, the pump had been cleaned with distilled water and methanol. The flow-through cell was disassembled and rinsed with distilled water after sampling from each well. The pump screen was also rinsed with distilled water and methanol between monitoring wells. According to recent sampling, the downgradient well 8 was less contaminated than 3, so 8 was sampled first to minimize any effects of cross contamination.

Due to the extensive time required to sample each well, and accessibility, well 16 was sampled using a bailer. The dedicated Waterra could not be used because the pump was not available and the depth of the water table (30 m) made it difficult to pump by hand. The Waterra tubing was removed from the well to allow purging. The flow-through cell was placed in a pail and parameters were measured as water was slowly poured into the cell from the bottom of the bailer. Once the measurements were stable, sample were drawn up in the bailer and carefully poured from the bottom into a 4-L jug. A sample of water was tested for DO using a Chemets Kit as described above.

Table 5-2. Summary of Sample Method and Pumping Information

MW	Sample Method	Volume Purged	Rate	Pump Pressure	Refill Time	Discharge
8	Bladder Pump	14 L	0.3 L/min	90 psi	20.3s	30.2s
3	Bladder Pump	16 L	0.5 L/min	90 psi	15s	27s
16	Bailer	8 L				

5.3.2.2 Aerobic Microcosm Experiments

The same method outlined in Section 5.3.2.1 was used to collect groundwater for aerobic experiments in May 2001. Well 3 was sampled with a bladder pump, 15L of water was purged at a pump rate of 0.5 L/min. Twelve litres of groundwater was collected in 4-L bottles.

5.3.3 GROUNDWATER SAMPLING AND ANALYSIS FOR METHANE

Groundwater samples to analyze for methane were collected in 40-mL vials (EPA Certified Clean Ltd) using a bladder pump as previously described. Water was gently added down the side of the bottle so as not to agitate or create bubbles, which could strip the methane. Each bottle was completely filled and a few drops of 6N hydrochloric acid were added as a preservative before capping. Samples were transported in a cooler to the University of Alberta for analysis.

Groundwater methane samples were measured by gas chromatographic headspace analysis with a Hewlett Packard 5700A GC with a flame ionization detector (FID) fitted with a 2 m x 0.3 cm column packed with Tenax GC (60/80 mesh). N_2 was the carrier gas at 50 mL/min, and H_2 and air were set at 35 mL/min and 300 mL/min respectively. Chromatographs and peak areas were obtained using a HP 3380A integrator (Holowenko et al., 2000). A calibration curve for methane was prepared by injecting three different known concentrations of methane into the GC in triplicate (Appendix C). Headspace samples were compared to the calibration curve to determine the concentration of methane in each groundwater sample.

To generate headspace in the sample vial, the bottle was placed upside down in a clamp attached to a ring stand. A 20 gauge needle attached to a syringe was inserted through the septum. Then, another 20 gauge needle attached to Teflon® tubing was also inserted through the septum. The Teflon® tubing was in turn attached to a nitrogen tank, which was set at a flow rate of 5mL/min or less. The nitrogen forced water out of the bottle into the syringe until 4 mL of water had been extracted. The needles were then removed and the bottle was shaken for 5 minutes to allow the gases to equilibrate between headspace and liquid phases (Kampbell and Vandegrift, 1998).

A gastight syringe was used to withdraw 1 mL of gas from the headspace of the sample vial. The headspace sample was then injected into the GC and compared to the calibration curve to determine the concentration of methane. Each well had triplicate samples. The concentration of CH_4 was calculated as described by Kampbell and Vandegrift (1998):

$$C = MW\left[\frac{55.5}{H} + \frac{V_g}{V_w}\frac{1}{22.4}\frac{273}{(T+273)}\right]X_g \qquad [24]$$

MW = molecular weight of gas [CH_4 = 16 g/mol and CO_2 = 44g/mol]

H =Henry's constant for gas [CH_4 = 412300 @ 25°C]

V_g = Volume of headspace

V_w = Volume of liquid

T = Temperature [°C]

X_g = Partial pressure of the gas (concentration detected by GC)

All data and calculations to determine methane and carbon dioxide concentrations can be found in Appendix C.

5.4 BART™ TEST

Prior to setting up an extensive microcosm study, it was necessary to quickly confirm the presence of biological activity in the groundwater. The Biological Activity Reaction Test (BART™) developed by Dr. R. Cullimore at University of Regina were used to detect the activity and types of microorganisms in the subsurface. BART™ is a patented test currently sold by Droycon Bioconcepts Inc. (DBI) in Regina.

The BART™ method was originally developed to assess biological activity associated with nuisance bacteria in water wells. The method is based on relating the lag time before evidence of biological activity is noted to the activity (aggressivity) of the bacteria responsible. There are several selective tests that have been developed to assess the various bacterial groups. Three types of BART™ were used to detect microbial activity at the study site:

• DN-BART™	denitrifying bacteria (DNB)
• IRB-BART™	iron-related bacteria (IRB)
• SRB-BART™	sulfate reducing bacteria (SRB)

- DN-BART™ - The presence and aggressivity of DNB is indicated by the lag time before development of foam and bubbles (nitrogen) around the ball at the top of the test vial. The absence of bubbles indicates DNB are not present.

- IRB-BART™ - There are a number of iron-related bacteria detected by this test. Bacterial presence is indicated by development of foam and/or reddish to brownish colouration (throughout the test vial or around the ball). A negative response is indicated by the absence of these features. Bubble formation around the ball suggests anaerobic activity.

- SRB-BART™ - The presence of SRB is demonstrated by the development of blackening around the vial base and/or the ball at the top of the vial. SRB are not present if there is no blackening in the vial.

Appendix D contains the interpretation sheets provided by DBI of each BART™. Detailed information is available through Droycon (2000).

The main limitation of using the BART™ method to provide supporting evidence for NA is that the test only provides an estimate of potential bioactivity. The method will identify the potential for activity of a specific bacterial type, but does not directly measure whether hydrocarbon biodegradation by that type of microorganism is occurring.

According to DBI, the BART™ approach offers two major advantages for use as a bioactivity test compared to more traditional agar plate techniques. First, the tests are easy to use and read. Secondly, the test kits can detect a variety of microbes from different environments. As long as appropriate field sampling and test set-up methods are used, this test method is simple enough that it can be used in the field.

Upstream (well 16), downstream (well 8) and within the plume (well 3) samples were tested in duplicate using DN, IRB and SRB BART™ types. A groundwater sample (10 mL) was transferred into each BART tube using a sterile widemouth pipette. The tubes were sealed and observed for reactions every 24 hours over a period of 10 days. They were kept at a constant temperature 10°C, which is similar to the groundwater temperature at the study site (8°C).

5.5 MICROBIAL ENUMERATION

Population densities of various microorganisms in soil and groundwater can be estimated by the most probable number (MPN) technique. This method consists of replicate test tubes containing serially diluted samples, which are monitored for the presence or absence of bacterial growth. The various types of microorganisms being enumerated must be able to have a detectable reaction. Growth or positive tests on a specific product are recorded throughout the incubation time and statistical analysis is utilized to determine the extent of bacterial growth in the subsurface (Alef and Nannipieri, 1995).

Samples were extracted from the 4-L bottles of groundwater collected from the study site. Groundwater from monitoring wells 16, 3 and 8 were used for enumeration. Tenfold serial dilutions from 10^0 to 10^{-7} were prepared and added to various liquid media designed to grow the following types of microbes:

- Denitrifying bacteria (DN)
- Sulfate-reducing bacteria (SRB)

- Iron-reducing bacteria (IRB)
- Total heterotrophs
- Hydrocarbon degraders

Each medium contained a specific product to selectively encourage the growth of a specific class of microorganisms. For example, the hydrocarbon degraders were enumerated on a mineral salt medium with filter-sterilized free-product from the study site. All five liquid media recipes are in Appendix E.

All the dilution bottles (10^0-10^{-7}) were dosed with 96 mL of 0.1% sodium pyrophosphate (a dilution buffer). Dilution buffer as opposed to distilled water is used to prevent drastic changes in pH and maintain the ion balance. The dilution bottles and rubber stoppers were then sterilized by autoclaving at 120°C for 1 hour. Thus the final volume of the dilution buffer was approximately 90 mL. Media for iron reducers (B10 medium) and total heterotrophs (R2A medium) consisted of sloppy agar, which was autoclaved and dispensed while still hot. Ten millilitres of medium were dispensed into each test tube (about 50 test tubes per medium) using aseptic technique. All other types of media were dispensed into test tubes (10 mL per test tube) and then sterilized by autoclaving. For the analysis of DN, an inverted Durham vial was added to each test tube prior to sterilization to monitor the production of N_2 gas. Two ¾" bright finishing nails were added to the SRB media. The media were allowed to cool overnight.

Dilution of the groundwater was carried out in a fumehood using aseptic technique. For each series of dilutions, ten millilitres of the groundwater sample was transferred to a dilution bottle using a sterile widemouth pipette. The dilution bottle was mixed thoroughly by inverting several times. Ten millilitres were then transferred to the second dilution bottle with another sterile widemouth pipette. The serial dilution process was repeated until all 7 dilutions were completed. One millilitre of each dilution was aseptically transferred to each of five replicate test tubes of medium and covered with sterile caps. This was repeated for each of the five media. Duplicate negative controls that contained no sample were included for each type of media. Duplicate positive controls were prepared by inoculating each medium type with 1 mL of a microbial culture that represented the bacterial group being enumerated. The test tubes were incubated at 10°C for 6 weeks, and scored weekly for positive results.

After 6 weeks, an additional test was carried out on test tubes containing the DN media to assess whether nitrate, nitrite or N_2 was the dominant product. Approximately 5 drops each of α-napthylamine in 5N acetic acid and sulfanilic acid in 5 N acetic acid, were added to each DN test tube. The appearance of a pinkish colour indicated the presence of nitrite. If there was no colour change then either all the nitrate had been converted to N_2 or was still in the form of nitrate. To check if there was any nitrate still present, a few grains of zinc powder were added. This chemically reduces nitrate to nitrite inducing a colour change and confirming that the nitrate had not been completely reduced to N_2.

Indicators of viable microbes in each medium were determined from the positive controls. The presence of IRB was indicated by a colour change to black or green at the base of the sloppy medium. SRB were detected by the presence of black precipitate (iron sulfides) on the nails added to each test tube. Heterotrophic bacteria were present if there were a significant number of flocs in the sloppy media compared to the negative controls. Turbidity and flocs associated with the free-product signalled the presence of hydrocarbon degraders. Reduction of nitrate to N_2 gas by DN bacteria was indicated by gas bubbles in the inverted Durham tubes. Nitrate reduction to nitrite by denitrifiers was revealed if the sample changed colour with the addition of chemicals described above.

After 6 weeks, the final positive tubes were tabulated. The MPN and confidence intervals were calculated based on the characteristic number and the dilution level (Alef and Nannipieri, 1995). The characteristic number was determined as follows. The first step was to select the tubes in the least concentrated dilution where all the tubes had a positive reaction or in which the greatest number of tubes were positive. Secondly, the number of positive tubes in the next two higher dilutions was recorded. For example, if the highest number of positive reactions is in 5 test tubes at a dilution of 10^{-3}, then the number of positive tubes at 10^{-4} and 10^{-5} were also recorded. In this case the characteristic number might be 5-4-3. The MPN would be obtained by using the characteristic number and the appropriate dilution in the statistical tables used in Alef and Nannipieri (1995). The 95% confidence intervals can also be determined from these tables. A sample calculation can be found in Appendix J.

5.6 ANAEROBIC MICROBIAL ACTIVITY

A number of techniques were used to assess the potential biodegradation rate of diesel from the site under anaerobic and aerobic conditions. The groundwater table at the site is more than 30 m below ground surface, and previous analysis had observed anaerobic conditions. The majority of experiments were set-up to mirror the in-situ environment. The rate of anaerobic mineralization was measured by monitoring increases in by-products (carbon dioxide and methane) and decreases in contaminant substrate (TEH). Use of electron acceptors by microorganisms was also measured by monitoring decreases in sulfate and nitrate under anaerobic conditions.

5.6.1 ANAEROBIC MINERALIZATION

A radiorespirometric microcosm laboratory study was performed to determine the potential for diesel biodegradation at the study site, to determine whether electron acceptor or nutrient amendment would increase the rate of degradation, and to quantify the effect on the rate. The mineralization of a known amount of radiolabelled dodecane, toluene or acetate was monitored to calculate the rate of biodegradation by indigenous microorganisms. The radiolabelled hydrocarbon was initially added to 125 mL serum vials (microcosms) containing groundwater from the contaminated area (well 3). The production of radiolabelled $^{14}CO_2$ in the microcosms gives direct evidence of microbial mineralization of the radiolabelled hydrocarbon. Monitoring the production of $^{14}CO_2$ over time gives the rate of biodegradation of diesel contamination at the study site.

Diesel fuel is a composite mixture of compounds including *n*-alkanes, branched and cyclic alkanes and aromatics. To determine the biodegradation rate of diesel contamination it was necessary to use a compound that was dominant in the diesel mixture. A semi-volatile GC/MS open scan conducted by Maxxam Analytics identified dodecane to have the highest percent area (2.9%) of the top ten compounds identified in the chromatogram. Furthermore, dodecane was readily available as a radiolabelled compound, thus appeared to be the appropriate choice to monitor diesel degradation. Radiolabelled toluene was used in a set of microcosms because it is one of the more soluble and easily degradable aromatic hydrocarbons. It has also been well studied in many environments and provides a baseline for comparative purposes. A set of positive controls was also prepared consisting of the contaminated groundwater and radiolabelled acetate, which is an easily biodegradable substrate. This provides a positive measure of microbial activity and gives confidence in the data obtained from the dodecane and toluene microcosms.

A number of experimental conditions were tested to analyze the biodegradation rate and extent of mineralization of diesel contamination under in-situ conditions. Some conditions also assessed the potential effects of amendment in enhancing the degradation process. The following microcosms were prepared (all radiochemicals are from Sigma Chemical Co.):

- Unamended at 10°C with Dodecane-1-^{14}C
- Unamended at 23°C with Dodecane-1-^{14}C
- Nutrient amended at 10°C with Dodecane-1-^{14}C
- Sulfate amended at 10°C with Dodecane-1-^{14}C
- Nitrate amended at 10°C with Dodecane-1-^{14}C
- Unamended at 10°C with Toluene-methyl-^{14}C
- Unamended at 10°C with Acetate-1-^{14}C

Specimens were prepared for triplicate destructive sampling over sixteen time events for each experimental condition. Negative controls for each type of radiolabel were sterilized by autoclaving for 1 hour. Acetate and toluene radiolabelled negative controls were set-up for every second time interval and dodecane negative controls were prepared for every time point. Table 5-3 provides a summary of the anaerobic microcosms sampling plan.

Serum vials and composite Teflon®-lined butyl stoppers were prepared for the microcosms by autoclaving several days prior to the experimental set-up. In addition, the amendment solutions were prepared and autoclaved, then added to the 125 mL serum vials prior to adding the contaminated groundwater. Each serum vial received 0.75 mL of a resazurin dye (0.1 g/L) and bicarbonate (100g/L) solution. Resazurin indicates the redox potential of the groundwater; resazurin is colourless when the potential is ≤ -200mV and pink at potentials more positive than -100 to -120 mV (Salanitro, 1997). However, the intermediates of denitrification (NO and N_2O) can also oxidize resazurin and turn cultures pink (Suflita et al., 1997). The bicarbonate is used to buffer the groundwater because the production of CO_2 may acidify the samples. Each sulfate and nitrate amended vial received 0.75 mL of 3 mM sodium sulfate (426 mg/L Na_2SO_4) solution or 3 mM of potassium nitrate (303 mg/L KNO_3) solution respectively. Each nutrient amended vial received 0.15 mL of sterile nutrient stock. All amendment recipes can be found in Appendix E.

All radioisotopes were kept on ice during sample preparation to prevent volatilization. The 4-L sample jugs were opened in the fumehood as they were needed and a canula was placed on the bottle to continuously flush the headspace with $10\%CO_2/90\%N_2$ gas to prevent excess oxygen entering the groundwater samples. The gas was passed over a heated copper column to remove excess oxygen and sterilize the gas flow prior to entering the serum vials. The headspace of the 125mL serum vials was also flushed with the same gas during inoculation and capping. Seventy-five millilitre aliquots of the contaminated groundwater were aseptically transferred to the serum vials using a 25mL widemouth pipette.

Table 5-3. Summary of Anaerobic Microcosm Experimental Set-up

			Time Steps								
Type	**Radiolabel**	**Temp.**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Negative Control	Dodecane	10	1	1	1	1	1	1	1	1	
Negative Control	Toluene	10	1		1		1		1		
Negative Control	Acetate		1		1		1			1	
Unamended	Toluene	10	3	3	3	3	3	3	3	3	
Unamended	Dodecane	10	3	3	3	3	3	3	3	3	
Nutrient amended	Dodecane	10	3	3	3	3	3	3	3	3	
Sulfate amended	Dodecane	10	3	3	3	3	3	3	3	3	
Nitrate amended	Dodecane	10	3	3	3	3	3	3	3	3	
Unamended	Dodecane	20	3	3	3	3	3	3	3	3	
Unamended	Acetate	10	3	3	3	3	3	3	3	3	
Total Samples			24	22	24	22	24	22	23	23	
Type	**Radiolabel**	**Temp.**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**Totals**
Negative Control	Dodecane	10	1	1	1	1	1	1	1		15
Negative Control	Toluene	10		1		1		1		1	8
Negative Control	Acetate	10		1		1		1		1	8
Unamended	Toluene	10	3	3	3	3	3	3	3	3	48
Unamended	Dodecane	10	3	3	3	3	3	3	3	3	48
Nutrient amended	Dodecane	10	3	3	3	3	3	3	3	3	48
Sulfate amended	Dodecane	10	3	3	3	3	3	3	3	3	48
Nitrate amended	Dodecane	10	3	3	3	3	3	3	3	3	48
Unamended	Dodecane	20	3	3	3	3	3	3	3	3	48
Unamended	Acetate	10	3	3	3	3	3	3	3	3	48
Total Samples			22	24	22	24	22	24	22	23	**367**

Each vial was dosed with the appropriate radiolabel using an Eppendorf® pipette set to the required sample volume, then capped and crimped with a composite Teflon®-lined butyl stopper (Fisher Scientific). The acetate microcosms were dosed with a 750 µl filter sterilized sample of radiolabelled acetate containing 42,000 disintegrations per minute (dpm). The serum vials that required radiolabelled toluene were given a 20 µl sample (dissolved in non-radiolabelled toluene) which contained 67,300 dpm. The dodecane radiolabel was dissolved in methanol and 20 µl containing 776,100 dpm was added to each of the remaining sample vials. Methanol as a carrier has also been used in other studies (Coates et al., 1997). Radiolabel was added to the negative controls after autoclaving to prevent volatilization. The microcosms were then incubated in the dark at 10°C and room temperature (23°C). The incubator at 10°C was monitored with a datalogger equipped with a thermister so the temperature would not significantly vary.

The collection of $^{14}CO_2$ was accomplished by selecting vials at random, and acidifying each vial with 2mL of 4N H_2SO_4 to convert bicarbonate to carbon dioxide then flushing the headspace for 15 minutes with N_2 gas at 100mL/min. The purged headspace then passed through a CO_2 trapping apparatus (Figure 5-1) containing 3 liquid scintillation vials. The acidified samples were kept in crushed ice before and during the flushing process to minimize volatilization of any radiolabel not converted to $^{14}CO_2$. The first liquid scintillation vial in series contained 10 mL of ACS fluor (Amersham) to capture any unconverted radiolabel (*i.e.* dodecane) carried by the nitrogen gas. The second and third vials contained 10 mL of ACS fluor and 1 mL of Carbasorb® (Packard), to trap radiolabelled CO_2. The ^{14}C activity was determined using a liquid scintillation counter (Beckman LS 3801) (description in Appendix F).

Initially, the 125 mL serum vials were subsampled at one time interval then completely flushed at the next sampling event. Using a 23 gauge syringe, 1.5 mL of liquid medium and 1 mL of headspace were extracted and injected into a chilled 38 mL serum bottle containing 2 mL of 4N H_2SO_4. The sample was then flushed for 5 minutes in the same manner as the whole microcosm. Subsampling was terminated after the fifth sampling point due to possible leaks in the stoppers after subsamples were extracted.

To test the CO_2 flushing apparatus for leaks and find a suitable flushing time for the 125 mL vials, test samples with a known quantity of $H^{14}CO_3^-$ was flushed through the system. The test sample was acidified with 2 mL of 4N H_2SO_4 to convert the bicarbonate to carbon dioxide. The recovery rate of the radiolabelled bicarbonate was determined by comparing the ^{14}C activity of the same volume (1 mL) that was added to the sample. This procedure was followed prior to every sampling event to test the flushing apparatus for leaks.

5.6.1.1 Dodecane Extraction

After more than 200 days of incubation a sequential solvent extraction was carried out to perform a mass balance to evaluate the amount of radiolabelled dodecane and toluene remaining in the anaerobic microcosms. After flushing, the remaining dodecane was extracted from microcosms using dichloromethane (DCM). Ten mL of DCM was added to each bottle using a 10 mL pipette. The sample was recapped and shaken for five minutes, then left to settle. The entire contents of the serum vial were poured into a separation funnel. The serum vial was rinsed with DCM, which was added to the separation funnel. In addition, the separation funnel was also rinsed with DCM to ensure all the radiolabelled dodecane or toluene was extracted. The DCM was then allowed to settle to the bottom of the flask, then the DCM was drawn off into a volumetric test tube. The amount of DCM used was recorded and a Pasteur pipette was used to extract a small amount into a dram vial. One millilitre of the extract was sampled using an Eppendorf® pipette and placed into 10 mL of fluor. The extract was then counted using the scintillation counter. This process was repeated with a second extraction. After both extractions were completed, 1 mL of water from the extracted sample was sampled using an Eppendorf® pipette and placed into 10 mL of fluor. This was also counted in the scintillation counter.

To quantify the amount of radiolabel that may have sorbed into the stoppers, the stoppers of each sample were chopped up into ~5 mm pieces and placed in test tubes containing 5 mL of ethyl acetate for 4-7 days. The ethyl acetate was then sampled using an Eppendorf® pipette, and 1 mL was injected into 10 mL of fluor, then counted. This method was carried out on May 8-14th, 2001. However, this did not prove highly effective, so stoppers of subsequent samples were chopped up using scissors and placed into 10 mL of fluor. The stoppers were allowed to sit in the fluor for 2-4 days prior to being counted on the scintillation counter because this allowed time for the radioactive compounds to enter the fluor from the stoppers. This method was carried out on September 24th-27th, 2001.

Dodecane extraction via sequential extraction did not yield the radiolabelled dodecane initially added to the microcosms. Recovery of radiolabelled dodecane was generally less than 5%. This is explained further in Section 6.7.1.1. Sorption to the stoppers, co-evaporation with methanol and volatilization were proposed causes. To evaluate the loss mechanism(s) of radiolabelled dodecane, extraction of ^{14}C-dodecane was conducted on replicate abiotic microcosms using distilled water. Radiolabelled dodecane dissolved in either methanol or filter sterilized free-product was added to 75 mL of distilled water in a 125 mL serum bottle. Extraction was immediately carried out with DCM as described above. Radiolabelled dodecane dissolved in methanol had a relatively low recovery (34-53%) whereas radiolabelled dodecane dissolved in free-product had a greater recovery (85-100%). Therefore it was deduced that addition of free-product would better facilitate the radiorespirometric experiments as well as providing additional organic carbon for the microorganisms.

5.6.1.2 Free-Product Addition

The lack of observed $^{14}CO_2$ evolution indicative of anaerobic degradation in the 125-mL microcosms after 8 months of incubation prompted a re-evaluation of the experiments. It was deduced that the microorganisms may not have had enough available carbon, so it was decided to spike the remaining microcosms with filter sterilized free-product from the study site. One triplicate set of each experimental condition was not spiked and put aside. The remaining microcosms were divided into two groups. The first group received 5 µL of free-product (53 mg/L) and the second group received 5 µL of radiolabelled dodecane (143, 000 dpm) dissolved in free-product.

5.6.1.3 Incubation

Addition of only free-product to the 125 mL microcosms did not improve $^{14}CO_2$ recovery. Hence, it was decided to use these bottles to determine whether radiolabelled dodecane was escaping via volatilization. The bottles were spiked with 5 µL of radiolabelled dodecane (143,000 dpm) dissolved in free-product and incubated upside down to prevent the headspace from contacting the stopper. After 4 weeks of incubation the bottles were flushed and extracted as previously described in Sections 5.6.1 and 5.6.1.1. In addition, sterile controls were prepared and spiked with 5 µL of radiolabelled dodecane (143,000 dpm) dissolved in free-product. These were incubated right side up at 10°C and 35°C to determine whether radiolabel was escaping through the stoppers.

5.6.2 MICROBIAL ACTIVITY – QUANTIFYING END PRODUCTS BY GAS CHROMATOGRAPHY

Anaerobic microcosms described in Section 5.6.1 were periodically measured for methane (CH_4) by gas chromatographic headspace analysis with a Hewlett Packard 5890 GC containing a packed column (2m stainless steel, Poropak R, 80/100 mesh) and a thermal conductivity detector. The carrier gas was high-purity helium at 23 mL/min. A HP 3390A integrator was used for signal acquisition and peak integration. A calibration curve for methane was prepared by injecting three different known concentrations of methane into the GC in triplicate (Appendix G).

After acidification with 2 mL of 4N H_2SO_4, a 1 mL headspace sample was extracted from each microcosm with a gas tight syringe and directly injected into the GC. Once GC analysis was complete, the samples were flushed as described in section 5.6.1. The CH_4 concentration was calculated as described in Section 5.3.3. Appendix G contains all data and calculations to determine methane and carbon dioxide concentrations.

5.6.3 MICROBIAL ACTIVITY - UTILIZATION OF ELECTRON ACCEPTORS

In parallel with monitoring $^{14}CO_2$ evolution, the consumption of nitrate and sulfate was also assessed as an indication that the microorganisms in the groundwater were actively degrading hydrocarbon contamination. A number of experimental conditions were prepared to test the consumption rate of nitrate and sulfate, both unamended and amended:

- Unamended at 10°C
- Unamended at 20°C
- Nitrate amended at 10°C
- Sulfate amended at 10°C
- Nutrient amended at 10°C

The microcosms were prepared in a similar manner in 125 mL serum vials (75 mL groundwater and 50 mL headspace) as described in Section 5.6.1, except no radiolabelled substrate was added. Six time events were prepared for each set of microcosms with triplicate incubations for each time point. The sampling frequency was to depend on the results from the radiorespirometric experiments. The serum vials were incubated in the dark at 10°C and room temperature (20°C). Sterilized negative controls were also prepared for each time interval.

The groundwater samples were analysed for sulfate and nitrate in the Limnology laboratory in the Biological Sciences Department at the University of Alberta. The methodology and quality control used in these analyses is described in detail in Appendix H.

5.6.4 MICROBIAL METABOLITES

Groundwater samples were sent to the University of Oklahoma in Norman, Oklahoma for hydrocarbon metabolite analysis. The 1-L samples were acidified with 5 mL of 50% HCl to pH 2 prior to shipment. The methods used to analyze for possible metabolites in the diesel contaminated groundwater are summarized below. Detailed methods can be found in Gieg et al. (1999b), Kropp et al. (2000), and Elshahed et al. (2001).

On arrival, samples were extracted with ethyl acetate, then concentrated on a rotary evaporator. The extracts were then derivatized with *N,O*-bis(trimethylsilyl)trifluoroacetamide (Pierce Chemicals Co., Rockford. IL) to form trimethylsiyl (TMS) esters. The components in the extracts were then analyzed by GC-mass spectrometry (MS) for the presence of known microbial metabolites of aerobic and anaerobic biodegradation. When possible, metabolite identifications were made by comparing GC retention time and MS profiles with authentic standards (Gieg and Suflita, 2001).

5.6.5 CHEMICAL ANALYSIS BY MAXXAM ANALYTICS, INC.

The production of end-products and consumption of terminal electron acceptors, such as nitrate and sulfate are powerful indicators that the mineralization of the parent substrate has occurred (Suflita et al., 1997). However, this analysis should be supported with other indicators of biodegradation. Apparent lack of mineralization may not necessarily mean that the parent substrate is recalcitrant, but that partial transformation of the substrate may result in forming products that are resistant to microbial biodegradation. Therefore, it is important to directly monitor the degradation of the contaminant substrate(s).

In this study the contaminants of interest were the components of diesel including TEH, TPH and BTEX. These substrates and the electron acceptors nitrate and sulfate were analyzed by Maxxam Analytics, Inc in the prepared groundwater sample. There were several logistical difficulties in preparing the microcosms for substrate analysis. Primarily, a minimum of 500 mL of groundwater was required for TEH analysis. Therefore, microcosms had to be prepared in 1-L sterilized glass amber bottles. There were 6 separate time event samples prepared for each series of microcosms, with duplicate samples for each sampling event. The experimental conditions for the large microcosms included:

- Unamended at 10°C
- Unamended at 20°C
- Nutrient amended at 10°C
- Sulfate amended at 10°C
- Nitrate amended at 10°C

Sterilized negative controls were also prepared by autoclaving for 1 hour. Prior to the addition of groundwater, all the necessary amendments were added to each 1-L bottle. Each bottle received 5 mL of a resazurin dye (0.1 g/L) and bicarbonate (100g/L) solution. Each sulfate and nitrate amended vial received 5 mL of 3 mM sodium sulfate (426 mg/L Na_2SO_4) solution or 3 mM of potassium nitrate (303 mg/L KNO_3) solution respectively. Each nutrient amended vial received 1 mL of sterile nutrient stock. The same amendment solutions were used as in Section 5.6.1.

The 4-L samples obtained in the field were homogenized in an autoclaved plastic 20-L container. A fitting on the bottom of the container allowed water to be poured into 1-L bottles. Approximately 500 mL of groundwater was added to each 1-L microcosm bottle directly from the 20-L container by carefully pouring the water down the side of the bottles to prevent aeration. After addition of groundwater, the bottles were flushed for 15 minutes with 10%CO_2/ 90%N_2 gas (passed over a copper column) to create an anaerobic headspace. The headspace of the 20 L container was also continuously flushed during sample preparation. The 1-L bottles were then capped with Teflon®-lined screw caps and sealed with parafilm and tape to minimize the entry of oxygen. The bottles were stored at 10°C in a BOD incubator in the Environmental Engineering building, and at 20°C in an incubator in the Newton building.

The time zero data analysed at Maxxam Analytics, Inc found the TEH concentrations too low to effectively track biodegradation. Thus, the remaining 1-L bottles were spiked with 25 μl (40 mg/L) of filter sterilized free-product. During addition of free-product, the bottles were flushed with 10%CO_2/ 90%N_2 gas (passed over a copper column) to minimize infiltration of oxygen.

Details on chemical analytical methodologies by Maxxam Analytics, Inc. can be found in Appendix B. TEH in the groundwater were extracted with carbon disulfide using heptane as a surrogate component. The extract was then analyzed using a gas chromatograph with a flame ionization detector (GC/FID). Purgeable hydrocarbons and BTEX were first separated into components using a temperature programmable gas chromatograph. The separated components were then identified and quantified by mass spectrophotometry. Both sulfate and nitrate were analyzed by colourimetric measurement using a spectrophotometer.

5.7 AEROBIC MICROBIAL ACTIVITY

Aerobic mineralization was also examined to determine the difference in degradation rate when oxygen is present. The purpose of aerobic radiorespirometric experiments was to determine the effects of oxygen on diesel biodegradation rates. Mineralization of radiolabelled dodecane to $^{14}CO_2$ was measured to find the degradation of diesel by indigenous microorganisms. The effects of temperature and nutrients addition were also examined. The following experimental settings were tested:

- Unamended at 10°C with Dodecane-1-^{14}C
- Unamended at 28°C with Dodecane-1-^{14}C
- Nutrient amended at 10°C with Dodecane-1-^{14}C

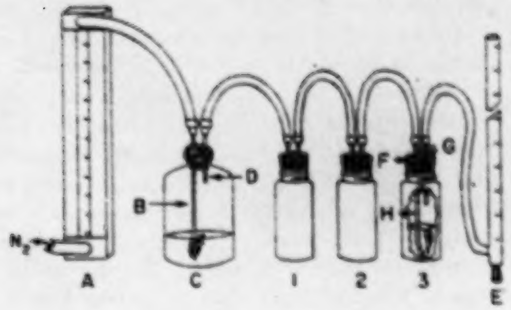
- Nutrient amended at 28 °C with Dodecane-1-^{14}C
- Positive Control at 10°C with Dodecane-1-^{14}C

All experiments were prepared in triplicate (except the positive control) and single negative controls were prepared for 10°C and 28°C. A total of 16 biometers were prepared.

The 4-L samples containing groundwater samples were opened in the fumehood. Using a widemouth pipette, 50 mL of sample was transferred to each modified 250mL Erlenmeyer flask, which had a test tube arm (Figure 5-2). All biometers received 20 µL of radiolabelled dodecane dissolved in free-product (252292 dpm). The nutrient amended biometers received 0.1 mL of sterile nutrient stock (Appendix E). Known hydrocarbon-degrading microorganisms were added to the positive control.

Ten millilitres of 1N KOH was added to the test-tube arm of the biometers. The KOH trapped any $^{14}CO_2$ produced in the biometer. The stopper in the side arm had a needle inserted with a 5 mL Luer-Lok syringe attached to prevent leakage of the KOH, and tubing extended from the needle into the KOH. This allowed quick and easy sampling of the biometers. During sampling the 5 mL syringe was removed, air was injected using a 1 mL syringe to clear the tubing and ensure a sample was taken from KOH in the test tube arm. Every 5-10 days, 0.5 mL of KOH was sampled from each biometer. When KOH reached 4-4.5 mL, more 1N KOH was added to the test tube arm. A sample calculation in Appendix N outlines the conversion of dpm to %$^{14}CO_2$.

The 0.5 mL of KOH sample was injected into a scintillation vial containing 10 mL of ACS fluor and 1.5 mL of double distilled water. The KOH is basic, so adding 0.1 mL of glacial acetic acid neutralized the solution. The blank scintillation vial (to determine background radioactivity) contained 1.5 mL of water, 10 mL of ACS fluor, 0.5 mL of sterile 1N KOH and 0.1 mL of glacial acetic acid. The scintillation vials were allowed to dark adapt for 15-20 minutes prior to being counted in the scintillation counter.



A = Flow regulator on N_2 tank
B=Inflow - 22 Gauge x 8.9 cm needle to allow N_2 bubbling in acidified sample
C = Serum vial (125 mL)
D = Outflow – 26 Gauge x 1.27 cm needle allows CO_2 to be carried into scintillation vials (bottles 1-3)
E = Soap bubble flowmeter to check N_2 flow rate
F = Modified scintillation caps
G = Outflow - 18 Gauge x 3.8 cm needles inserted through cap to allow CO_2 to be carried to the next scintillation vial
H = Inflow - 22 Gauge x 8.9 cm needle inserted through cap to bubbles inflow into fluor and allows CO_2 to enter each vial

Figure 5-1. Microbial Activity Apparatus (after Fedorak et al., 1982)

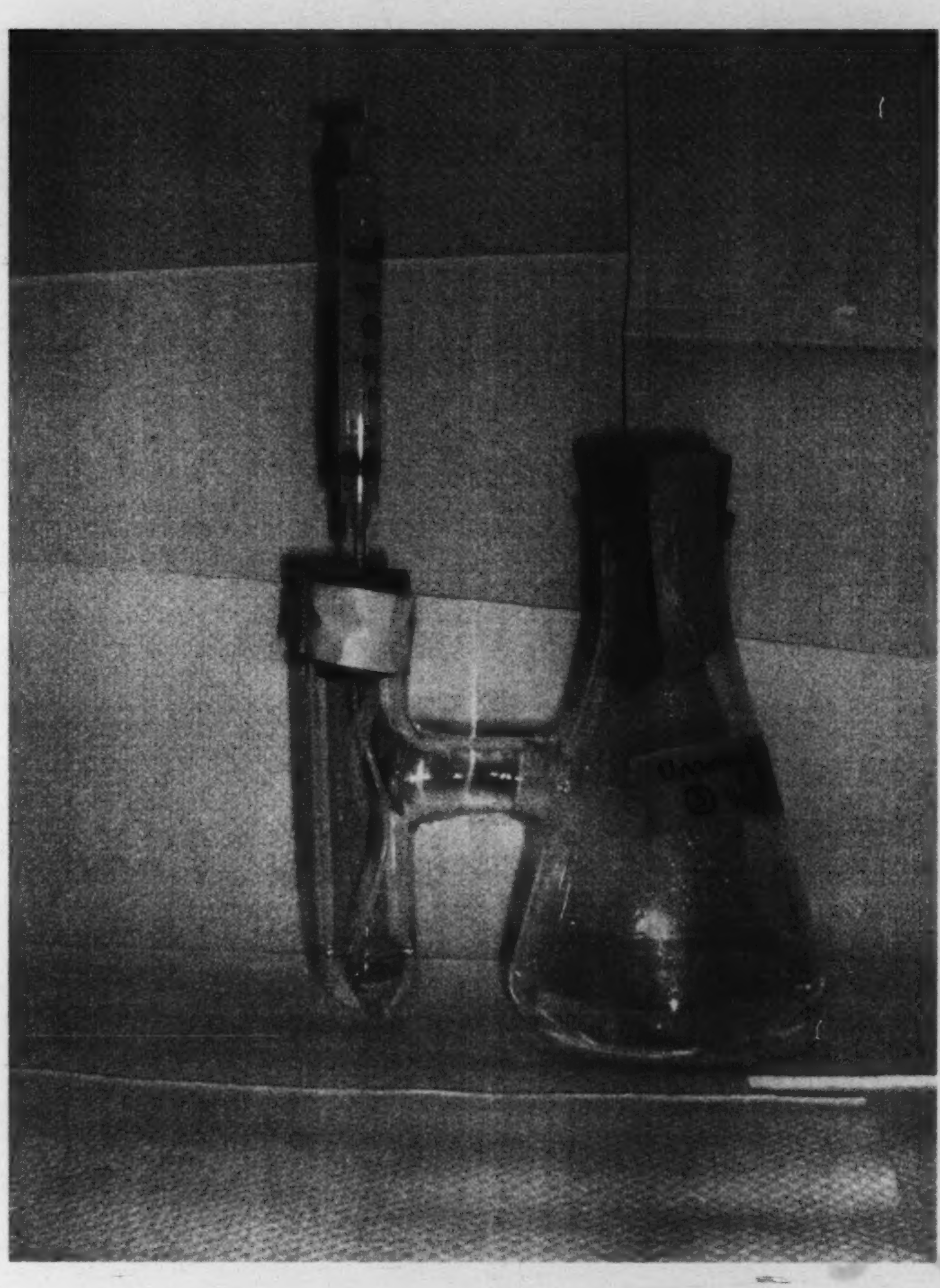


Figure 5-2. Photograph of Biometer Experiments

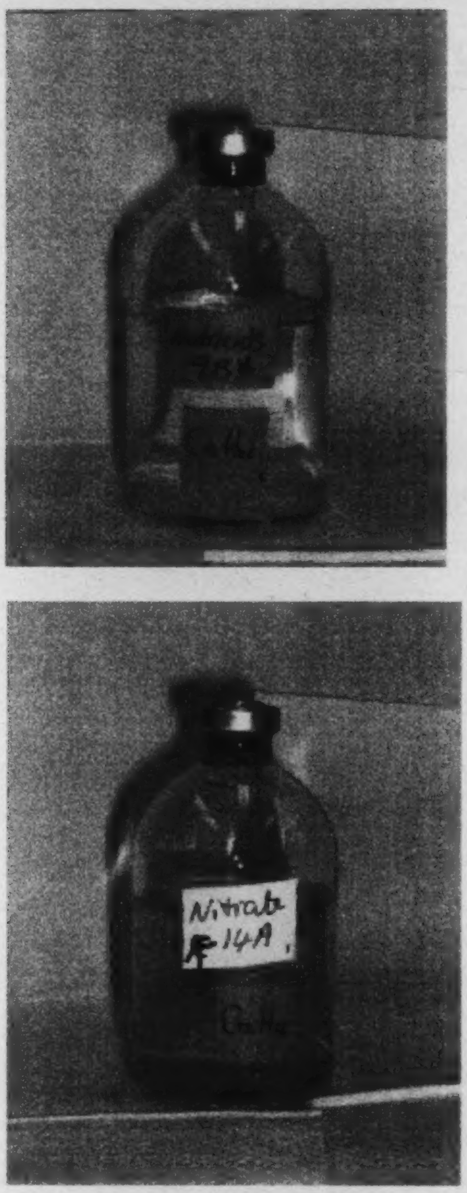


Figure 5-3. Photographs of Nutrient Amended (clear) and Nitrate Amended (pink) Microcosms Containing Radiolabelled Dodecane

6. CASE STUDY - RESULTS AND DISCUSSION

6.1 INTRODUCTION

Results from the study site field sampling and laboratory experiments are presented and discussed in this chapter. The data gathered are used to support the three lines of evidence for NA discussed in Chapter 2. This includes historical groundwater chemistry data, hydrogeological and geochemical data, and microbiological studies.

A description of remediation activities at the site is provided in Section 2.4, and a description of the local hydrogeology is in Section 5.2. A review of the subsurface conditions at the site is provided below, followed by results and discussion of field measured data. Next, the results from microbial enumeration are presented and discussed. Finally the results of aerobic and anaerobic laboratory microcosms are analyzed and discussed.

6.2 HISTORICAL DATA

The characteristic components of diesel fuel include *n*-alkanes, (n-C_9 to n-C_{27}), paraffinic, aromatic and olefinic hydrocarbons (Bregnard et al., 1996). Therefore TEH, which includes hydrocarbons ranging from C_{11}–C_{30} provides a reasonable measure of dissolved diesel concentration. Diesel degradation may be slower than BTEX because of the presence of heavier hydrocarbons (Borden, 1994).

Table 6-1 and Table 6-2 show concentration changes over time of TPH and TEH respectively, from a selection of downstream, plume and upstream monitoring wells. A complete set of hydrocarbon data for all monitoring wells at the study site can be found in Table A-4, Appendix A

Table 6-1. TPH Concentration (mg/L) Over Time at the Study Site

Location	MW	Distance from source (m)	July 1996	Nov 1996	Nov 1997	May 1998	Aug 1998	Jan 2000	Sept 2000	April 2001
Upstream	16	-50				<0.0012			<0.0012	<0.0012
Plume	2	75	0.012	<0.0008	<0.0008	<0.0012	<0.0012	0.0058	<0.0012	<0.0012
Plume	3	195	1.34	0.253					0.92	1.292
Downstream	8	560		<0.0008	<0.0008	<0.0012	<0.0012	<0.0005	<0.0012	<0.0012
Downstream	19	690				<0.0029	0.0007		<0.0012	<0.0012

Table 6-2. TEH Concentration (mg/L) Over Time at the Study Site

Location	MW	Distance from source (m)	July 1996	Nov 1996	Nov 1997	May 1998	Aug 1998	Jan 2000	Sept 2000	April 2001
Upstream	16	-50				<0.02			<0.6	<0.6
Plume	2	75	15.2	3.14	0.42	<0.02	0.57	1.1	<0.6	1.1
Plume	3	195	7.79	4.56					26.5	
Downstream	8	560		0.61	<0.01	<0.02	<0.02	0.55	<0.6	<0.6
Downstream	19	690				<0.02		0.5	<0.6	<0.6

Analysis of contaminants indicates that the dissolved phase hydrocarbon plume (consisting primarily of TEH) has migrated in an east-southeast direction 400 to 700 m from the well site source (Komex International Ltd., 2001a). The free-phase hydrocarbon plume extends 400 m from the source (Komex International Ltd., 2001a). TPH concentrations are generally below the detection limit, except at well 3, which is in the centre of the plume. Data from well 3 indicate that TPH concentrations are stable and TEH concentrations appear to have increased (Table 6-1 and Table 6-2). However, sampling events are separated by 4 years and there is a high possibility of free-product contaminating the sample because the well has free-product floating atop the water table.

The measurable TEH plume (Figure 6-1) is larger than the free-product (

Figure 6-2) or BTEX plumes. Between 1996 and 2001 the TEH plume length varied between a minimum of 400 m to a maximum of 700 m from the source. Downgradient monitoring wells have apparent increases and decreases of TEH concentrations between monitoring events, which is likely dependent on method detection limits, groundwater surface fluctuations and possibly contamination of samples with free-product while sampling. Also, seasonal or periodic variations in recharge and groundwater elevations may cause significant fluctuations in contaminant concentrations and in monitoring wells for successive sampling events (Kennedy et al., 2001). However, the overall dimensions of the dissolved hydrocarbon plume are relatively stable since the initiation of monitoring in 1996 (Komex International Ltd., 2001a).

Throughout the monitoring period from 1996 to the present, BTEX compounds at well 3 have often been above the Canadian Drinking Water Guidelines (CDWG). Locations where guidelines have previously been exceeded were reported at wells 13 and 4 in 1996 and 1997. Groundwater in well 4 is still likely to be contaminated above guideline values because it is in the plume and often has free-product floating on the surface. In April 2001, concentrations of BTEX compounds were above the guidelines at wells 3, 20 and 21 (Table 6-3). There are no drinking water quality criteria for TPH and TEH.

Table 6-3. BTEX Concentrations at the Study Site[1].

MW CDWG	Benzene 0.005 mg/L	Toluene 0.024 mg/L	Ethylbenzene 0.0024 mg/L	Xylenes 0.3 mg/L
3	0.0012	0.029	0.031	1.22
20	0.0081	0.0059	0.0085	0.280
21	0.0069	0.0040	0.0073	0.0517

1 – Grey coloured cells indicate concentrations exceeding CDWG

Free-product has been detected at wells 3, 4, 13 and 28. The length of the free-product plume has remained relatively consistent over the monitoring period, however there is temporal variation in free-product thickness. For example, the apparent free-product thickness at well 3 has varied between 0.039 m (minimum) to 0.39 m (maximum), with an average of 0.2 m.

Figure 6-2 shows the distribution and thickness of free-product on the groundwater table at the study site. A photograph of free-product extracted from well 3 is shown in

Figure 6-3.

6.3 FIELD MEASURED PARAMETERS

Values of pH, electrical conductivity, redox potential and temperature for all monitoring wells can be found in Table A-2 of Appendix A.

The pH of groundwater can impact the three-dimensional conformation of enzymes and transport proteins of microbial cells. This affects a microorganism's ability to conduct cellular functions, cell membrane transport, and the equilibrium of catalyzed reactions (Suthersan, 1997). Most microorganisms have evolved with pH tolerances of 5 to 9, but prefer 6.5 to 7.5. This is within the range of pH values found in most natural environments (Suthersan, 1997). Measured values of pH at the study site ranged from 6.5 to 8.1, with an average of 7.4. This indicates the groundwater is neutral and within optimal conditions.

Electrical conductivity is a measure of the ability of a solution to conduct electricity (Wiedemeier et al., 1995a). The concentration of ions in groundwater is directly related to electrical conductivity; as ion concentration increases so does conductivity. Electrical conductivity measurements at a site are used to ensure that groundwater samples are from the same saturated zone (Wiedemeier et al., 1995a). Electrical conductivity in groundwater at the study site ranged from a minimum of 309 μS/cm to 905 μS/cm, with a mean of 580 μS/cm. Conductivity at the majority of wells tended to be close to the mean.

Redox potential measures whether an environment is oxidizing or reducing. Ranges of redox potential in the natural environment range from +800 mV, when concentrations of O_2 are constantly elevated, to –400 mV, when H_2 concentrations are high (Suthersan, 1997). Redox measurements at the study site vary greatly from year to year within and between wells. In general, wells that are within or close to the plume have redox potentials of approximately –20mV. According to Table 3-2, this is an indication of iron reduction. However, there appears to be little iron available at the site, and geochemical indicators discussed in Section 6.4.2 support sulfate reduction and methanogenesis. Therefore, redox potential measurements at this site do not appear to be a reliable method of evaluating the electron accepting conditions in the groundwater.

When sampling with a flow-through probe there is the possibility of an increase in groundwater temperature especially if the air temperature is warm. All historical temperature measurements, except in October, 2000 and May, 2001, were conducted using a down-hole probe. The average winter temperature is 4.9°C, whereas the average fall and spring temperatures are 1°C higher at 6.2°C and 6.1°C respectively. Average summer temperatures show a further increase to 7.7°C. Temperatures at well 3 (where groundwater was extracted for microcosms) ranged between 5.5°C and 8°C. The temperature ranges at the study site indicate that microbial communities are likely to be psychrotolerant. This means that the microorganisms have the ability to grow at low temperatures and their optimum temperature for growth is greater than 15°C (Bej et al., 2000).

6.4 GEOCHEMISTRY

Table 6-4 shows a summary of contaminant and geochemical values from selected monitoring wells at the site. Table A-4 and Table A-3 in Appendix A have the complete set of contaminant and geochemical data. Methane concentrations can be found in Appendix C. Additionally, Appendix A contains a detailed chemical analysis of groundwater from wells 8 and 3.

Table 6-4. Summary of Contaminant and Geochemical Data

Location	MW	Distance (m)	BTEX (mg/L)	TEH (mg/L)	TPH (mg/L)	DO (mg/L)
Background	16[1]	-50	ND	ND	ND	3
Plume	2[1]	75	ND	1.1	ND	0.5
Plume	3[2]	195	1.26	26.5	0.82	ND
Plume	4[3]	365	0.16	4.37	0.07	ND
Downstream	8[1]	560	ND	ND	ND	0.3
Downstream	27[1]	800	ND	ND	ND	4.8
Location	**MW**	**Distance (m)**	**NO_3^- & NO_2^- (mg/L)**	**SO_4^{2-} (mg/L)**	**Mn (II) (mg/L)**	**CH_4 (mg/L)[4]**
Background	16[1]	-50	0.271	7.9	0.068	ND
Plume	2[1]	75	0.026	6.8	0.041	NA
Plume	3[2]	195	ND	0.3	0.413	13.3
Plume	4[3]	365	0.004	0.9	0.151	20.2
Downstream	8[1]	560	0.006	11.8	0.024	10.7
Downstream	27[1]	800	0.321	37.6	0.005	NA

[1] Measurements taken on April 25th, 2001
[2] Measurements taken on April 25th, 2001 (BTEX), and September 19th, 2000 (all others)
[3] Measurements taken on November 6th, 1996
[4] Methane samples taken May 22nd, 2001
ND = Non-detect (below detection limit)
NA = Data not available (parameter was not measured)

6.4.1 CONTAMINANT CONCENTRATION TRENDS

TEH concentrations range from 28 mg/L in the plume to non-detectable levels downgradient and upgradient (Figure 6-1). Hydrocarbon concentrations were elevated within the plume, however the highest values occurred 195 m from the source. Lower TEH and BTEX measurements closer to the source may be due to the variability caused by the plume migrating through fractured bedrock. The location of a monitoring well may not be in an area where contaminants have spread into the fractures. The free-product plume is laterally discontinuous, as it does not appear in some wells in the interpreted plume area. Contamination that has seeped into the fractured bedrock may be sequestered within the rock matrix, on fracture coatings, in NAPL zones, or within poorly-connected fractures (US EPA, 2001). These all provide potential sources of future contamination, which require ongoing remediation.

Table 6-5 lists hydrocarbon compounds detected in the groundwater that fall in the TEH range. Dodecane has the highest percent of total area (2.9%) from the chromatograph. Although this area does not directly correlate with the concentration of dodecane (due to lack of a reasonable standard for the weathered diesel), it does indicate that it is one of the most abundant compounds. Mohn et al. (2000) assumed that dodecane constituted 3.3% of total petroleum hydrocarbons from contaminated sites.

Table 6-5. Top Ten Peaks from a Semi-Volatile Open Scan of Groundwater from Well 3.

Peak Number	Component	% of Total Area
1	Dimethylbenzene	2.6
2	Tridecane	2.5
3	Tetradecane	2.5
4	Trimethylbenzene (Isomer)	1.5
5	Dodecane	2.9
6	Pentadecane	1.9
7	Methyltridecane	2.3
8	Undecane	1.6
9	Trimethylbenzene (Isomer)	1.2
10	Heptadecane	1.4

NB: Naphthalene, 2-methylnaphthalene, fluorene and phenanthrene were also identified as a small percentage of the total chromatographic area.

6.4.2 GEOCHEMICAL INDICATOR TRENDS

Geochemical data can be used to determine which mechanisms of intrinsic bioremediation are operating at the study site, as well as the relative importance of each mechanism (Wiedemeier et al., 1995b). Analysis of geochemical changes in this study included measuring the depletion of TEAs and increase in by-products. The TEAs analyzed were dissolved oxygen (DO), nitrate (NO_3^-) and nitrite (NO_2^-), and sulfate (SO_4^{2-}). Analysis of by-products included iron (II), manganese (II) and methane. The changes in concentration of TEAs and by-products from upstream through the plume to downstream are presented in Table 6-4.

DO within the plume is depleted to non-detectable levels, whereas concentrations upstream and downstream measurement are 3.0 and 4.8 mg/L respectively. The contour map of DO (Figure 6-4), illustrates the depletion of oxygen within the TEH plume area. Comparison with Figure 6-1 demonstrates that areas with elevated TEH have depleted DO concentrations. Low DO extends further downstream beyond the TEH plume indicating that there has been active use of DO as a TEA.

NO_3^- and NO_2^- concentrations steadily decrease from upstream (0.271 mg/L) to plume wells (ND) and then increase in wells directly downstream (0.321 mg/L).

Figure 6-5 confirms the depletion of NO_3^- and NO_2^- in the plume area as well as directly downstream. However, NO_3^- and NO_2^- concentrations are relatively low compared to other TEAs and the amount of hydrocarbon metabolized would be very small (Section 3.2.2), thus denitrification is not likely a significant TEAP at this site.

Dissolved iron concentrations were typically below the detection limit. Thus, it was concluded that iron (III) was likely not an applicable TEAP at the study site. This is reasonable in fractured bedrock, since the bioavailable forms of iron (III) discussed in Section 3.2.3 are less prevalent than in a soil matrix. Dissolved manganese concentrations appeared to be at a maximum (0.413 mg/L) within the plume and close to non-detect further downstream at well 27 (0.005 mg/L). Although these elevated Mn(II) concentrations (Figure 6-6) generally correspond closely with the dissolved hydrocarbon plume, the low concentrations indicate Mn(IV) is a minor TEA.

Sulfate concentrations were depleted within the plume (0.3 mg/L) relative to background (7.9 mg/L) and downstream concentrations (37.6 mg/L) (Table 6-4). Sulfate is greatly depleted in the centre of the plume at wells 3 and 4, where TEH concentrations are highest (Figure 6-7). The relatively large change in concentration between background and plume wells, indicates that sulfate reduction is likely a major TEAP at the study site.

Methane concentrations were non-detectable upstream and downstream of the contaminated area, and high levels of methane were found within the plume (up to 20 mg/L). Methane was also detected downstream at a concentration of 9.5 mg/L at well 19. It is possible that the dissolved methane detected in the groundwater is natural gas that may have migrated from the bedrock formation. However, methane concentrations are only elevated in areas where there is hydrocarbon contamination, or directly downstream (Figure 6-8). Detectable methane concentrations extend beyond the TEH plume boundaries, which may be due to transportation of dissolved methane or may result from biological degradation of non-TEH organic microbial metabolites. It is common for methane to be produced in an actively methanogenic zone, and carried by flowing groundwater to non-methanogenic zones. Methane concentrations measured at a monitoring well may not be indicative of methanogenesis in the immediate area, but might be occurring upstream (Chapelle, 2001). The high levels of methane produced in the contaminated area indicate that methanogenesis is an active biodegradation process as dissolved hydrocarbons migrate downstream in the aquifer

6.4.3 MASS BALANCE

Radiolabelled dodecane was used in anaerobic and aerobic mineralization experiments to determine the level of microbial activity at the study site. The following balanced equations describe dodecane degradation using various TEAs:

Dissolved Oxygen: $C_{12}H_{26} + 18.5O_2 \Rightarrow 12CO_2 + 13H_2O$ **[25]**

Nitrate: $C_{12}H_{26} + 14.8NO_3^- + 14.8H^+ \Rightarrow 12CO_2 + 20.4H_2O + 7.4N_2$ **[26]**

Mn (IV) $C_{12}H_{26} + 37MnO_2 + 74H^+ \Rightarrow 12CO_2 + 37Mn^{2+} + 50H_2O$ **[27]**

Fe (III): $C_{12}H_{26} + 74Fe(OH)_3 + 148H^+ \Rightarrow 12CO_2 + 74Fe^{2+} + 198H_2O$ **[28]**

Sulfate: $C_{12}H_{26} + 9.25SO_4^{2-} + 18.5H^+ \Rightarrow 12CO_2 + 13H_2O + 9.25H_2S$ **[29]**

Methanogenesis: $C_{12}H_{26} + 5.5H_2O \Rightarrow 2.75CO_2 + 9.25CH_4$ **[30]**

The total amount of TEH within the plume during the most recent sampling event was between 41.9 and 26.5 mg/L. The lower measurement is used because there was a possibility of free-product contaminating the sample. Equations 24 to 29 were used to determine the EBC of each TEAP. Table 6-6 summarizes the EBC for each TEAP at the study site. The concentration change was calculated by taking the difference between concentrations of electron acceptors or by-products within the plume (wells 3 or 4) and upstream (well 16) as reported in Table 6-4. The EBC assumes that each biodegradation reaction goes to completion, and does not take into account the formation of metabolites. Additionally, it was assumed that no substrate was taken up as biomass (Wiedemeier et al., 1999).

Table 6-6. Summary of Electron Biodegradation Capacity (mg/L) at the Study Site

	Mass of dodecane degraded(mg) per mg of TEA used	Mass of dodecane degraded(mg) per mg of by-product produced	Conc. Change (mg/L)	EBC (mg/L)
Oxidation	0.29		3.00	0.86
Denitrification	0.19		0.27	0.05
Iron Reduction		0.04	0.00	0.00
Manganese Reduction		0.04	0.35	0.01
Sulfate Reduction	0.19		7.60	1.46
Total EBC				2.38
Methanogenesis		1.15	20.00	22.99

Methanogenic bacteria use the by-products from other aerobic and anaerobic biodegradation reactions, such as acetate, H_2 and carbon dioxide. Therefore, the EBC from methanogenesis represents the total EBC for both anaerobic and aerobic biodegradation mechanisms. Assuming that dissolved methane can be used to calculate the total EBC of dodecane, then 23 mg/L of dodecane can potentially be degraded to CO_2 and H_2O at the site. The total EBC from all other TEAPs is 2.38 mg/L. The large difference in these two total EBC indicates methanogenesis is an important TEAP at the site.

The EBC of approximately 23 mg/L does not exceed the maximum measured concentration of 41.5 mg/L (well 13). It is assumed that dodecane degradation reactions represent all TEH compounds in the groundwater. However, Table 6-5 shows compounds that range from C_8 to C_{17}. Each of these compounds and other trace contaminants will have different EBCs. The presence of heavier hydrocarbons may decrease the overall EBC so that the concentration of TEH further exceeds the EBC at the site. Despite an inadequate biodegradation capacity to mineralize the maximum level of TEH within the plume, it is expected that the EBC may be sufficient to biodegrade hydrocarbon in wells on the periphery of the TEH plume as the contaminants are diluted. This may prevent dissolved TEH migrating further downstream. However, further modelling would confirm whether contaminants are effectively removed by biodegradation as they are transported downstream.

6.4.4 GIBBS FREE ENERGY

Energy given out by a reaction is not a measure of its driving force (Sawyer et al., 1994). When a chemical reaction occurs under isothermal conditions, a portion of the energy is lost because of the tendency of entropy to increase, and the remaining energy is termed Gibbs free energy (ΔG) (Gaudy and Gaudy, 1980). It should be noted that (ΔG) is not a physical term but a concept to determine what processes will occur spontaneously and which reactions are thermodynamically favourable.

ΔG for a reaction can be determined from Gibbs free energy of formation for each compound (G_f) involved in a reaction. The overall ΔG is found by subtracting the ΔG of the reactants from the products:

$$\Delta G = \sum_{products} n\Delta G_f - \sum_{reactants} n\Delta G_f \qquad [31]$$

where n is the number of moles of each compound. The values of ΔG and ΔG_f change with temperature and pressure, however, it is not necessary to know these values for each compound at all temperatures. ΔG at various temperatures can be determined from the Gibbs energy for the reaction at standard temperature and pressure (STP) (temperature =298.15 K, pressure = 1atm) together with the enthalpy of the reaction (Elliott and Lira, 1999). This concept was developed into a form of the van't Hoff equation:

$$\frac{\Delta G_{T_2}}{RT_2} = -\int_{T_1}^{T_2} \frac{\Delta H_{T_2}}{RT_2^2} dT + \frac{\Delta G^0}{RT_1} \qquad [32]$$

where ΔG^0 = Gibbs free energy at STP [kJ/mol]

ΔG_{T2} = Gibbs free energy at T_2 [kJ/mol]

ΔH_{T2} = Enthalpy of the reaction at T_2 [kJ/mol]

$R = 8.314 \times 10^3$ [kJ/Kmol]

T_1 = 298.15 K

T_2 = temperature at which the reaction occurred

Finding ΔH at T_2 can be difficult and time-consuming, so an approximation is made to assume that ΔH is independent of temperature, thus ΔH^0 at STP can be substituted for ΔH_{T2} in Equation 32. Values for ΔH^0 of a reaction were determined from the enthalpy of formation for each compound (ΔH^0_f). The overall ΔH^0 was found using the following equation:

$$\Delta H^0 = \sum_{products} n\Delta H^0_f - \sum_{reactants} n\Delta H^0_f \qquad [33]$$

Appendix I contains the calculated values of ΔH^0 for all contaminants and electron acceptors of concern. Integrating equation 32 with ΔH^0 held constant gives the shortcut van't Hoff equation:

$$\Delta G_{T_2} = -\Delta H^0 * T_2\left(\frac{1}{T_2} + \frac{1}{T_1}\right) + \frac{\Delta G^0 * T_2}{T_1} \qquad [34]$$

This equation provides rapid insight about the effects of temperature and is applicable to temperature ranges within +/-100 K (Elliott and Lira, 1999). A sample calculation to determine ΔGT_2 at 10°C is provided in Appendix I

Table 6-7. Comparison of ΔG^0 Values at STP (Wiedemeier, 1995a) to ΔG Values at 10°C.

	Benzene			Toluene		
	ΔG^0	G(10°C)	%*	ΔG^0	G(10°C)	%
Oxidation	-3202.0	-2867.6	10	-3823.0	-3423.3	10
Denitrification	-3245.0	-2928.9	10	-3875.0	-3497.3	10
Manganese Reduction	-3202.0	-2878.3	10	-3824.0	-3437.1	10
Sulfate Reduction	-514.3	-488.0	5	-597.7	-567.7	5
Methanogenesis	-135.6	-87.1	36	-142.6	-140.5	1

	Ethylbenzene			1,3 Xylene		
	ΔG^0	G(10°C)	%	ΔG^0	G(10°C)	%
Oxidation	-4461.0	-3994.5	10	-4448.0	-3982.8	10
Denitrification	-4522.0	-4081.1	10	-4509.0	-4069.4	10
Manganese Reduction	-4461.0	-3995.1	10	-4449.0	-3984.4	10
Sulfate Reduction	-697.7	-662.6	5	-685.6	-651.8	5
Methanogenesis	-166.7	-164.1	2	-154.6	-153.3	1

Table 6-7 continued

	1,2 Xylene			1,4 Xylene		
	ΔG^0	G(10°C)	%	ΔG^0	G(10°C)	%
Oxidation	-4451.2	-3985.8	10	-4451.0	-3985.6	10
Denitrification	-4512.4	-4072.6	10	-4512.2	-4072.4	10
Manganese Reduction	-4690.1	-4213.3	10	-4689.9	-4213.1	10
Sulfate Reduction	-688.3	-654.3	5	-688.1	-654.1	5
Methanogenesis	-156.8	-155.3	1	-156.6	-155.1	1

	Dodecane		
	ΔG^0	G(10°C)	%
Oxidation	-7953.7	-7118.1	11
Denitrification	-8061.6	-7271.1	10
Manganese Reduction	-7956.6	-7147.3	10
Sulfate Reduction	-539.0	-551.8	-2
Methanogenesis	-387.4	-369.2	5

* % Decrease in the value of ΔG° to ΔG(10°C)

Biodegradation reactions with higher free energy, such as oxidation and denitrification tend to have a larger decrease when temperature drops compared to lower energy reactions such as methanogenesis. All hydrocarbon compounds listed in Table 6-7 show a 10-11% decrease in ΔG from 25°C to 10°C for oxidation, denitrification and manganese (IV) reduction. All hydrocarbon compounds show a 5% decrease in ΔG for sulfate reduction, with the exception of dodecane which had a 2% increase in ΔG. Toluene and all the xylenes demonstrated a 1% decrease in ΔG for methanogenesis, ethylbenzene and dodecane had a 2% and 5% ΔG decrease respectively. However, benzene showed a 36% decrease in ΔG for methanogenesis, which indicates that this reaction becomes substantially unfavourable as the temperature decreases (Richmond et al., 2001).

Other environmental factors may affect ΔG at the study site. The values above are theoretical values under isothermal conditions, which is not the case in the subsurface. The ΔG values calculated simply give an indication of what reactions are likely to occur under colder temperatures.

6.5 MICROBIAL ENUMERATION

Complete microbial enumeration data can be found in Appendix J. Figure 6-9 illustrates the populations of microorganisms detected at the study site using the MPN method. The highest populations of microorganisms were found at the background well (16). Significant populations of total heterotrophs and aerobic hydrocarbon degraders were found at all wells. Hydrocarbon degraders were more than an order of magnitude greater upgradient compared to within the plume. This was accepted because hydrocarbon-degraders are generally ubiquitous in the environment (Margesin and Schinner, 1999). Additionally, hydrocarbon-degrading bacteria at pristine sites (such as the Antarctic) are known to adapt to their contaminated environment and develop the ability to degrade hydrocarbon contaminants (Atlas, 1995; Wardell, 1995). Both the hydrocarbon (JP-4) degrading MPN test and total heterotrophs were conducted under aerobic conditions, thus the low numbers of aerobic microorganisms within the plume is likely because the groundwater at the site is anaerobic.

Denitrifiers (reducing NO_3^- to N_2) range from 10^5/mL in the background well to 10^2/mL within the plume. Geochemical values show non-detect nitrate concentrations within the plume, however nitrate concentrations outside the plume are below 1 mg/L. The lower number of denitrifying microbes within the plume and low nitrate concentrations indicate that the groundwater is nitrate limited and an alternate TEAP is being used. Denitrifiers that were capable of reducing nitrate to nitrite were detected at all wells at a concentration of 10^7/mL. This indicates that there may be many denitrifiers in the groundwater that lack the ability to reduce nitrate to nitrogen gas.

Sulfate reducing bacteria were not detected in the MPN tests for any of the wells despite the positive results from the BART™ tests. Also, geochemical evidence strongly suggests that sulfate reduction is taking place within the plume and possibly downgradient at well 8. This phenomenon was also experienced by Johns (1999), and might be due to the MPN media or procedure.

6.6 BART™

The BART™ enumerations provided a rapid screening that was used to evaluate microbial activity in the groundwater. The results were correlated with other NA lines of evidence to determine the extent of microbial biodegradation in the groundwater. As previously mentioned in Section 5.4, BARTs™ were not designed for NA evaluation but rather to assess biological activity associated with nuisance bacteria in water wells (Drycon Bioconcepts Inc., 2000). This study was done to determine if they were potentially useful for NA assessment.

Positive results were observed in the IRB BART™ at all three wells (3, 8 and 16). However, geochemical measurements indicate that iron-reduction is not likely prevalent at the site. The DN BART™ also produced positive results but there appeared to be a low degree of aggressivity. This is reasonable given the low nitrate concentrations. Reaction in the SRB BART™ only occurred within the plume (well 3). The sulfate levels in the plume are considerably depleted, indicating active sulfate reduction. BART™ results from each well are summarized in Table 6-8. Complete results can be found in Appendix D.

Table 6-8. BART™ Responses at Upstream, Plume, and Downstream Monitoring Wells

		BART™		
MW	**Location**	**DN**	**IRB**	**SRB**
16	Upstream	✓	✓✓	
3	Plume	✓	✓✓	✓✓
8	Downstream	✓	✓✓	

✓ = low aggressivity
✓✓ = medium aggressivity

Armstrong et al. (2001) reviewed the correlation between BART™ results and geochemical indicator trends at a number of upstream oil and gas contaminated sites. The DN-BART™ tended to test positive with low to medium aggressivity, despite low nitrate concentrations. The test indicates that denitrifying bacteria are present, however does not reveal whether denitrification is a significant TEAP. IRB-BART™ results were similar between monitoring wells in this study. Armstrong et al. (2001) concluded that the response of this test was inconsistent with dissolved iron concentrations. This is because the IRB-BART™ responds to a complicated series of reactions. The IRB- BART™ tests for iron-related bacteria, thus this test does not just screen for Fe(III)-reducing microorganisms, but can detect aerobic and anaerobic iron-utilizing bacteria. SRB-BART™ results were consistent with hydrocarbon trends and geochemical data in this study and in analysis by Armstrong et al. (2001).

Thus the BART™ appears to support MPN analysis in confirming the presence of microbes but was not always accurate in elucidating the TEAPs at the study site. The DN- BART™ gave a more accurate representation of the low aggressivity of denitrifiers than the MPN test. The IRB- BART™ was a poor indicator of iron reduction as a TEAP. However, the SRB- BART™ gave a good indication of active microbial sulfate reduction, where the SRB MPN has consistently failed to accurately reflect this process in a number of NA investigations. The BART™ results also aided in designing the microbial activity microcosm experiments, in showing that addition of sulfate and nitrate could potentially increase the rate of degradation despite low temperatures.

6.7 MICROBIAL ACTIVITY

Microbial biodegradation of contaminants at the study site was examined under anaerobic and aerobic conditions via laboratory ^{14}C radiolabel microcosm experiments. The recovery of ^{14}C radiolabel in the anaerobic tests was problematic, so an additional investigation of loss mechanisms of ^{14}C was implemented. This section will first present the results of ^{14}C recovery experiments, followed by a review of anaerobic microcosm study results. Finally, the results of an aerobic ^{14}C radiolabel study will be presented and discussed.

6.7.1 ANAEROBIC DEGRADATION

Once fuel hydrocarbon contamination enters the groundwater system, rapid depletion of dissolved oxygen by microorganisms results in the establishment of anaerobic conditions within the contaminated area (Wiedemeier et al., 1995a). The groundwater from the study site is 30 m below the ground surface and dissolved oxygen was low to non-detectable levels in the contaminated area. The redox potential and other geochemical indicators suggest that the groundwater is highly reduced and anaerobic. Therefore, experiments were conducted under anaerobic conditions to reflect these *in-situ* conditions. Microcosms were incubated under a variety of conditions described in Section 5.6. Production of $^{14}CO_2$ was used as a measure of biodegradation rate.

After 211 days of incubation the anaerobic microcosms containing dodecane and toluene radiolabel still had low levels of ^{14}C recovery as $^{14}CO_2$. Thus, a mass balance was calculated to evaluate the amount of radiolabelled dodecane and toluene remaining in the serum vials.

6.7.1.1 Mass Balance – ^{14}C-Recovery from Dodecane

The methods used to recover remaining radiolabel are described in Section 5.6.1.1. A mass balance conducted on May 8th-14th, 2001 only recovered a small amount (1.4-6.2%) of the ^{14}C-dodecane initially added to the microcosms. The dodecane radiolabel could not be accounted for in the remaining water, the extract using DCM or the stoppers extracted with ethyl acetate. Table 6-9 contains the total percentage range of ^{14}C recovered from each set of microcosms. Only in the sterilized negative control could all the dodecane be accounted for. The majority of radiolabel recovered was found in the stopper. However, as there were no replicates for the negative control it is difficult to confirm whether or not this was an isolated incident. Data detailing distribution of % recovery from flushing, fluor, extraction, water and stoppers can be found in Appendix K.

Table 6-9. Total % Recovery of ^{14}C in Anaerobic Microcosms after 211 Days of Incubation (May8th-14th)

Sample	% ^{14}C Total Recovery (Range of 3 Samples)
Unamended 10°C	2.3-3.8
Unamended 20°C	4.3-5.5
Nutrient Amended	2.5-5.9
Nitrate Amended	1.5-5.8
Sulfate Amended	1.4-6.2
Negative Control	98.7

Due to the apparent loss of ^{14}C from the microcosms, an investigation was conducted to determine what happened to the initial addition of ^{14}C-dodecane. Discussion with other researchers produced suggestions that may provide reasons for radiolabel loss, which included:

a) Co-evaporation of ^{14}C-dodecane with the methanol carrier (Kropp, 2001; Christensen, 2001)

b) Lack of available hydrocarbon (Kropp, 2001)

c) Sorption into the caps (Kropp, 2001)

d) Sorption to the glass (Christensen, 2001; Morley, 2001)

e) Incorporation into biomass (Christensen, 2001)

f) Leakage from the bottles (Christensen, 2001; Kropp, 2001; Morley, 2001)

g) Production of methane that uses $^{14}CO_2$ (Davis, 2001; Morley, 2001)

Each of these possibilities was explored to determine where the radiolabelled ^{14}C might have disappeared. Appendix K contains detailed percent recoveries that are discussed below.

a) Co-evaporation

Co-evaporation of initial addition of ^{14}C-dodecane with the methanol carrier was examined by adding radiolabel (dissolved in either methanol or filter-sterilized free-product) to 75 mL of double distilled water in a 125 mL serum vial. Full extraction as described in Section 5.6.1.1 was then carried out immediately to recover the ^{14}C-dodecane. It should be noted, only Teflon® caps were used to minimize sorption to the stoppers. Recovery of ^{14}C-dodecane dissolved in methanol ranged from 32 to 53%, whereas recovery of ^{14}C-dodecane dissolved in free-product ranged from 80-91% recovery. This indicates that more than 50% of radiolabel may have co-evaporated when ^{14}C-dodecane was initially added to the anaerobic microcosms. Furthermore, during radiolabel addition the serum vials were flushed with 10%CO_2/90%N_2 gas during the additions of sample, amendments and radiolabel, which may have aided in the evaporation of the dodecane radiolabel. However, dodecane has a low vapour pressure so its disappearance may be due to other causes such as dissolution and partitioning of dodecane in free-product.

b) Lack of available hydrocarbon

Tracer studies can be extremely difficult when very small amounts of radiolabelled material are used, thus measuring biodegradation can be a challenge (Davis, 2001). The apparent loss of ^{14}C-dodecane in the anaerobic microcosms made hydrocarbon mineralization difficult to detect. Concentrations of dissolved hydrocarbon were approximately 3 mg/L in the microcosms. The low levels of dissolved hydrocarbon and lack of free-phase meant that the radiolabel does not easily dissolve in the groundwater and consequently is not readily available to microorganisms for mineralization. In other words, there was insufficient free-phase for the dodecane to adequately partition and remain bioavailable in the groundwater.

Concentrations of available hydrocarbon were increased by a number of additions to the remaining microcosms. The additions are outlined in Figure 6-10. On day 270, half of the microcosms were spiked with 5 µL of only free-product and the other half received 5 µL of free-product containing ^{14}C-dodecane. Addition of only free-product would potentially allow any remaining ^{14}C-dodecane to dissolve into the hydrocarbon phase. However, the addition of only free-product did not improve recovery (1.5 –14.8% total recovery), indicating that the majority of original ^{14}C-dodecane dissolved in methanol was no longer in the microcosms.

The ^{14}C-dodecane dissolved in free-product was fully recovered after incubation of 20 and 62 days in the majority of microcosms. Recovery ranged from 41 to 185%. The high recovery values initially appear anomalous, but are reasonable as explained below.

In Quality Control (QC) in commercial labs, acceptable percentage recovery from standards often falls in the range of 80-120%. Assuming that up to 120% of ^{14}C-dodecane dissolved in the recently added free-product could be recovered, anything above this value may be attributed to the original ^{14}C-dodecane addition (dissolved in methanol). Calculations to evaluate whether 120% recovery of the second addition of ^{14}C-dodecane plus remaining ^{14}C from the first addition are reasonable and are in Table K-3, Appendix K. Recovered radiolabel attributed to the initial ^{14}C-dodecane ranged from 0-13.6%. The initial radiolabel addition was mainly detected in samples that were incubated for 61-62 days. This potentially indicates that the remaining radiolabel required time to dissolve in the added free-product. Once dissolved, the initial radiolabel was easier to recover. Thus the high recovery values do appear plausible.

c) Sorption to stoppers

The possibility of sorption of radiolabel onto or into the composite Teflon®-lined butyl stoppers was examined by extracting ^{14}C-dodecane directly from stoppers that had been used to seal incubated microcosms. Stoppers were cut up into small pieces (~5 mm) and placed directly into a scintillation vial containing 10 mL of ACS fluor. Readings were taken at a variety of time intervals up to 7 days. It was found that detectable radioactivity increased when the stoppers were left in fluor for 2-4 days. The amount of ^{14}C-dodecane recovered from the stoppers generally ranged from 0-36%, with the dodecane sterile negative control stopper containing 97% of the original radiolabel. However, the majority of stoppers sorbed close to the average of 4.1% of ^{14}C-dodecane.

There may be potentially more radiolabel in the stoppers that cannot be extracted. However, this is unlikely because 100% of radiolabel could be accounted for in microcosms spiked with ^{14}C-dodecane dissolved in free-product.

Bregnard et al. (1996) found up to 12.8% of ^{14}C-naphthalene sorbed to butyl rubber stoppers. Although the stoppers in this study were Teflon®-lined, there is the possibility of butyl rubber being exposed and sorbing the radiolabel.

d) Sorption to glass

Dodecane is insoluble in water (3 µg/L) so has a tendency to sorb to other materials. Although the ^{14}C-dodecane may have sorbed to the glass and become unavailable for mineralization, it should have been recovered during the extraction process. Each bottle was rinsed twice with DCM.

e) Incorporation into biomass

Although there was no visible biological growth in the microcosms, there is a possibility that ^{14}C-dodecane may have become incorporated into biomass. Extraction with DCM does not recover ^{14}C-dodecane in biomass, so the remaining water was tested for radioactivity. Recovery from water ranged from 0 to 9.3%. Incorporation of ^{14}C-dodecane into biomass does not appear to be significant.

f) Leakage from bottles

The ^{14}C-dodecane may have potentially escaped from the microcosms by leaking through the stoppers. To determine whether ^{14}C-dodecane had escaped in this manner, sterile controls containing ^{14}C-dodecane dissolved in free-product were incubated at 10°C and 35°C for 3 weeks. If ^{14}C-dodecane was escaping through the stoppers then it was expected that recovery from the 35°C sterile control would be significantly lower than at 10°C. However, recoveries were found to be extremely close, 59.5% and 65.5% at 10°C and 35°C respectively.

Further testing involved microcosms that received only extra free-product were additionally spiked with ^{14}C-dodecane dissolved in free-product on day 304 (see flow-chart). These bottles were inverted, so that the vapour phase was not exposed to the stoppers, and incubated at 10°C. Recoveries in these microcosms were comparable to the microcosms spiked with ^{14}C-dodecane dissolved in free-product on day 207, which were not inverted. This suggests that leakage of vapour through the caps was insignificant.

During acidification and flushing of samples to recover ^{14}C-dodecane in the form of $^{14}CO_2$, a large proportion of ^{14}C-dodecane appeared to volatilize out of solution and dissolve in the first scintillation vial (containing only fluor). This may be an area of potential loss of ^{14}C-dodecane. However, the amount of ^{14}C-dodecane volatilization in microcosms that did not receive additional ^{14}C-dodecane was less than 2%. Therefore, it is unlikely that ^{14}C-dodecane was lost during acidification and flushing.

g) Methane production that may use $^{14}CO_2$

Methanogenesis appears to be a dominant TEAP in the groundwater at the study site. Production of $^{14}CH_4$ was therefore deemed a potential sink for the missing ^{14}C-dodecane (dissolved in methanol). Methane in the headspace was measured on a GC/TCD after the sample was acidified. Results are tabulated in Table 6-10. Prior to addition of extra free-product and radiolabel, methane concentrations in the microcosms did not exceed the negative control. This suggests that methane production was minimal, thus $^{14}CO_2$ loss due to methanogenesis was not a viable mechanism. The microcosms incubated at 23°C were an exception, where methane concentrations were approximately double the negative control. Although $^{14}CO_2$ may have been actively used as an electron acceptor, $^{14}CO_2$ recoveries from these microcosms were not any lower than the other experimental conditions. The concentration of methane in all the microcosms including the negative control appeared to be relatively high, however this could be explained by the concentration of 13.3 mg/L of dissolved methane in the groundwater at the source (well 3).

Methane production did appear to increase relative to the negative control after addition of ^{14}C-dodecane dissolved in free-product. Overall methane concentrations are likely lower from the August 8th sampling event because the bottles had been opened during additions of free-product only and dodecane radiolabel dissolved in free-product. Microcosms incubated at 23°C did appear to have an increase in methane relative to the negative control. After 61 days of incubation (with ^{14}C-addition), recoveries of ^{14}C-dodecane in the unamended samples at 23°C (41-88%) were lower in comparison to the other experimental conditions (Appendix K, Table K-2). Thus, $^{14}CO_2$ may have been used an electron acceptor in methanogenesis but the effect appears too small to be significant. More data are required to reach conclusive results.

Table 6-10. Methane Concentrations (mg/L) in the Headspace of Anaerobic Microcosms

Date (Year = 2001)	**March 26th**	**April 18th**	**May 11th**	**May 28th**	**August 8th**	**September 24th**
Type of addition	None	None	None	None	Extra dodecane and free-product	Extra dodecane and free-product
Sulfate 10°C	---	---	7.0	6.8	3.2	3.2
Nitrate 10°C	---	---	7.8	7.1	2.9	2.6
Nutrient 10°C	---	---	7.9	7.4	3.8	2.9
Unamended 10°C	---	7.1	7.6	5.5	2.5	1.8
Unamended 23°C	---	14.1	13.2	11.1	1.8	4.9
Negative Dodecane 10°C	---	7.9	8.9		1.8	1.4
Type of addition					Extra free-product	Extra dodecane and f-p, incubated upside down
Sulfate 10°C	---	---	---	---	3.9	0.0
Nitrate 10°C	---	---	---	---	3.3	0.0
Nutrient 10°C	---	---	---	---	4.8	1.7
Unamended 10°C	---	---	---	---	1.3	0.0
Unamended 23°C	---	---	---	---	3.2	0.0

--- = Methane was not measured

After examining each of these possibilities to determine where the radiolabelled ^{14}C-dodecane might have disappeared, the findings are inconclusive. However, it is possible that some of the unaccounted radiolabel sorbed to the stoppers because there was insufficient free-phase hydrocarbon available for the dodecane to partition into and become bioavailable to the microorganisms. There is also the possibility of co-evaporation during addition and loss during methane production.

6.7.1.2 Mass Balance - ^{14}C-Toluene Recovery

Recovery of radiolabelled toluene during sequential extraction ranged from 55-95%. The majority appeared to volatilize into the first fluor vial (6-60% recovery). The remainder was extracted as unconverted radiolabel and a small amount was found in the stoppers (2-8%). The recovery from $^{14}CO_2$ evolution ranged from 0.3 to 1.4%. These data can be found in Appendix K, Table K-2.

6.7.1.3 Trends in ^{14}C Utilization in Anaerobic Microcosms

Acetate is a readily biodegradable substrate that provides a positive measure of the activity of the subsurface microorganisms, thus, microcosms containing ^{14}C-acetate were used as a positive control. Approximately 63% of the radiolabelled acetate was converted to $^{14}CO_2$ (Figure 6-11). This demonstrates that there is anaerobic microbial activity in the groundwater.

Another set of microcosms contained methyl-^{14}C-toluene, which is often used in biodegradability studies. Measurement of toluene degradation allowed direct comparison with other anaerobic investigations. However, toluene biodegradation to $^{14}CO_2$ never exceeded 5% even when spiked with an additional carbon source (free-product) on day 270 (Figure 6-12). Although biodegradation of toluene under anaerobic conditions has been demonstrated in a number of studies (Evans et al., 1991a; Evans et al., 1991b; Edwards et al., 1992; Edwards and Grbic-Galic, 1994), the lack of anaerobic degradation of toluene in this study could mean that *in-situ* conditions do not favour mineralization of this compound. Long lagtimes for adaptation of microbial populations have been observed, and an incubation time of more than 300 days may not have been long enough to adapt the natural populations to this substrate (Linkfield et al., 1989; Edwards and Grbic-Galic, 1994).

Anaerobic biodegradation of ^{14}C-dodecane was difficult to interpret due to the loss of the initial ^{14}C-dodecane explained in Section 6.7.1.1. Even after extra radiolabel and free-product were added to the microcosms the effectiveness of anaerobic biodegradation could not be determined. Addition of just free-product had little effect in all microcosms. More data are required to determine whether anaerobic degradation is taking place. However, some patterns can be interpreted from the available data.

The nutrient amended anaerobic microcosms (with ^{14}C-dodecane) had the highest %$^{14}CO_2$ recovery (up to 16%) prior to any free-product or radiolabel additions (Figure 6-13). Nutrient concentrations in the groundwater at well 3 are typically low. Total phosphate was 0.003 mg/L, ammonia was 0.01 mg/L, and nitrite plus nitrate were <0.003 mg/L. Thus, nutrient addition may have stimulated microbial activity (see Appendix E for nutrient addition values). The addition of free-product and ^{14}C-dodecane dissolved in free-product did not appear to significantly increase activity so it is possible that these microcosms were not substrate limited. During analysis for methane using the gas chromatograph, both nutrient and nitrate amended microcosms produced N_2O peaks. The peaks increased in area as time progressed. This indicates active denitrification in these microcosms. There may be further reduction to N_2 gas, however further analysis is required to determine the extent and products of denitrification.

Anaerobic biodegradation in the nitrate amended microcosms never reached the same level of biodegradation as the nutrient amended bottles prior to day 270 (Figure 6-14). This implies that nitrogen (in the form of nitrate) may not be the only limiting nutrient. Degradation rates can often be limited by the available quantity of a key nutrient (Graham et al., 1999). In this case, the absence of phosphate may have severely limited anaerobic mineralization. However, after the addition of hydrocarbon on day 270, there appeared to be an increase in biodegradation, generating up to 30% $^{14}CO_2$ production. The nutrient amended microcosms were not as affected by substrate amendment, thus more data are required to produce reliable results as to whether the nitrate amended microcosms were substrate limited. It is possible the nitrogen amended microcosms were limited by a lack of ^{14}C-dodecane as well, as discussed previously.

Unamended microcosms at 10°C exhibited a similar behaviour after extra ^{14}C-dodecane dissolved in free-product was added (Figure 6-15) although to a lesser extent. This increased mineralization may indicate that anaerobic biodegradation is feasible under *in-situ* conditions when sufficient carbon is available. The possibility that oxygen entered the bottles when the hydrocarbon additions were conducted at days 270 and 304 is discussed below, but is deemed insignificant. However, the bottles were opened for less than 3 seconds and were flushed with 10%CO_2/90%N_2 gas. Furthermore, although the resazurin redox indicator turned pink when the bottles were opened, it became colourless in the majority of bottles after 3 weeks of incubation, and was colourless in all bottles after 4 weeks. Nitrate amended bottles remained pink because the redox potential is higher than other anaerobic TEAPs. The oxygen required to degrade approximately 12% of added hydrocarbon would have been 2 mg/L. It is unlikely that this amount entered the headspace and was dissolved in the water in 3 seconds while the headspace was being flushed with an anaerobic gas mixture.

Sulfate amended microcosms (at 10°C) and unamended microcosms at 23°C did not show an overall increase in biodegradation after the radiolabel and free-product additions (Figure 6-16 and Figure 6-17). Although some bottles did show increased mineralization, this was offset by low $^{14}CO_2$ recoveries in replicates.

There were large variations in percent recovery of $^{14}CO_2$ in all the microcosms after the additions of ^{14}C-dodecane dissolved in free-product. The majority of variation between replicate samples usually occurs in the log phase of growth, which may explain the variability between triplicates after the additions.

Although the sulfate-amended microcosms did not show significant mineralization of ^{14}C-dodecane (Figure 6-16), sulfate reduction was evident through other observations. Also, a black precipitate formed in the bottles, which is likely due to hydrogen sulfide formation and metal sulfide precipitation. Edwards et al. (1992) made similar observations in active sulfate-reducing microcosms.

The results from the anaerobic experiments imply that the conditions in the microcosms were not appropriate for anaerobic biodegradation of ^{14}C-dodecane. The apparent loss of the initial ^{14}C-dodecane also complicated results. A longer acclimation period may be required for the microcosms. Acclimation periods for contaminant compounds in anaerobic environments can range from 2 weeks to 6 months or longer (Linkfield et al., 1989). Geochemical data suggest that methanogenesis is an active TEAP in the subsurface at the study site. Edwards and Grbic-Galic (1994) found that long adaptation periods (100 to 200 days for toluene degradation and 200-255 days for *o*-xylene degradation) before biodegradation were observed under methanogenic conditions.

6.7.1.4 Geochemical and Contaminant Trends from Microcosms

Anaerobic microcosms (125 mL serum vials without radiolabel) were monitored for sulfate and nitrate depletion under a variety of conditions including sulfate, nitrate and nutrient amended, and unamended at 10 and 23°C as described in Section 5.6.3. The nitrate amended microcosms received 303mg/L of nitrate and the sulfate-amended bottles received 426 mg/L of sulfate. The nutrient amended samples contained nutrient stock described in Appendix E.

Table 6-11 contains a summary of nitrate and sulfate concentration changes over time in the various anaerobic microcosms. Complete results can be found in Appendix H. Nitrate was below the detection limit in the unamended groundwater (<0.003 mg/L), thus was only detected in the nitrate and nutrient amended microcosms. Nitrate concentrations steadily decreased from 167 to 126 mg/L in the nitrate amended microcosms. Nitrate concentrations also decreased slightly in the nutrient amended vials. This implies that nitrate reduction is a potential TEAP if sufficient nitrate is available to indigenous microorganisms. The population of denitrifiers detected by MPN further supports these results.

Sulfate concentrations decreased to non-detect in the unamended microcosms at both temperatures. Sulfate-reducers may have biodegraded available hydrocarbons using the small amount of available sulfate as an electron acceptor. This suggests that sulfate-reduction may still be an active TEAP in the groundwater. The sulfate-amended microcosms did not show a decrease in sulfate concentrations. However, the resazurin redox indicator turned colourless after 1-2 weeks of incubation and remained colourless throughout the experiments. Also, a black precipitate formed on the glass, which is likely to be a result of hydrogen sulfide formation and metal sulfide precipitation. Sulfate concentrations may have been too high to see a significant decrease over time.

Table 6-11. Nitrate and Sulfate Concentrations (mg/L) in Anaerobic Microcosms

Nitrate	01-Dec-00 (Day 10)	01-Mar-01 (Day 91)	16-May-01 (Day 168)	20-Sep-01 (Day 264)
Negative Control	0.00	0.00	0.00	0.00
Sulfate Amended	0.00	0.03	0.00	0.00
Nitrate Amended	169.83	131.53	129.44	125.70
Unamended (10°C)	0.00	0.00	0.00	0.00
Unamended (23°C)	0.00	0.00	0.00	0.00
Nutrient Amended	123.66	130.18	129.37	107.40

Sulfate	01-Dec-00	01-Mar-01	16-May-01	20-Sep-01
Negative Control	0.31	0.42	0.53	0.00
Sulfate Amended	337.44	335.66	330.98	322.03
Nitrate Amended	0.52	0.59	0.67	0.00
Unamended (10°C)	0.44	0.23	0.19	0.00
Unamended (23°C)	0.52	0.06	0.05	0.00
Nutrient Amended	0.39	0.56	0.67	0.49

Time Zero = November 21st, 2000

Large 1-L microcosms containing 500 mL of groundwater from well 3 were incubated under a number of conditions including sulfate, nitrate and nutrient amended, and unamended at 10 and 23°C. These microcosms were analyzed for changes in nitrate, sulfate, TEH and TPH concentrations. The complete data set containing chemical analysis of these microcosms is located in Appendix L.

The data at time zero reported TEH concentrations that were too low to effectively track biodegradation. This was unexpected because initial chemical analysis of the groundwater indicated that the TEH concentration was approximately 26-42 mg/L. A Waterra pump was used for sampling that may have allowed free-product to contaminate the initial samples sent for analysis, giving a false high TEH concentration. Groundwater for all the microbial experiments was extracted using a bladder pump, which is a low-flow pump that minimizes disturbance of water in the monitoring well. The two different methods of sampling produced appear to have contradictory TEH concentration results, however dissolved hydrocarbon concentrations are probably best represented by results from the bladder pump sampling. Although this means the level of dissolved hydrocarbons in the groundwater is quite low, the free-product provides a continuous source of contamination. It was difficult to measure changes in TEH concentrations so close to the detection limit, therefore the remaining 1-L bottles were spiked with 40 mg/L of filter sterilized free-product on November 16th, 2000, 16 days after the initial set-up.

Table 6-12 shows that TEH concentrations did not decrease from 40 mg/L in unamended microcosms incubated at 10 and 20°C. However, TEH concentrations in both nutrient and nitrate amended microcosms decreased substantially. Nitrate (initially 303mg/L) appeared to be used as an electron acceptor in both nutrient and nitrate amended bottles. This reveals that the groundwater is likely nutrient deficient. Sulfate (initially 426 mg/L) concentrations appeared to decrease in the unamended samples. There was no apparent sulfate concentration change in the nutrient and nitrate microcosms because nitrate was probably being used as an electron acceptor. Sulfate did seem to decrease in the sulfate-amended microcosms, however more data are required to support this observation. A black precipitate appeared in the sulfate-amended samples as previously noted in other sulfate enriched microcosms.

Table 6-12. Nitrate, Sulfate, TEH and TPH Concentrations in 1-L Anaerobic Microcosms Incubated under a Variety of Conditions.

TEH (mg/L)	14-Nov-00	09-Jul-01	06-Sep-01
Negative Control	<2	44.5	45.4
Unamended 10°C	<2	57.0	41.5
Unamended 20°C	<2	42.5	46.7
Nutrient Amended	<2	29.5	27.1
Nitrate Amended	<2	36.7	31.7
Sulfate Amended	<2	50.1	40.4

TPH (mg/L)	14-Nov-00	09-Jul-01	06-Sep-01
Negative Control	<0.1	0.2	0.65
Unamended 10°C	0.2	0.2	0.75
Unamended 20°C	0.25	<0.1	0.7
Nutrient Amended	0.25	<0.1	0.3
Nitrate Amended	0.15	<0.1	<0.1
Sulfate Amended	0.25	0.05	<0.1

NITRATE (mg/L)	14-Nov-00	09-Jul-01	06-Sep-01
Negative Control	0.0765	0.013	0.0075
Unamended 10°C	<0.003	<0.003	<0.003
Unamended 20°C	<0.003	<0.003	<0.003
Nutrient Amended	29.5	15.5	1.8
Nitrate Amended	42.2	24.5	21.0
Sulfate Amended	<0.003	<0.003	0.0025

SULFATE (mg/L)	14-Nov-00	09-Jul-01	06-Sep-01
Negative Control	<1	0.2	0.65
Unamended 10°C	<1	0.45	0.1
Unamended 20°C	<1	0.6	0.1
Nutrient Amended	<1	0.55	0.5
Nitrate Amended	0.9	0.5	1.9
Sulfate Amended	320.0	144.2	261.5

Bottles were spiked with 40 mg/L of free-product on November 16th, 2000.

6.7.1.5 Microbial Metabolite Analysis

Dr. Gieg at the University of Oklahoma conducted microbial metabolite analysis on groundwater samples taken from well 3. Based on GC retention times and mass spectral (MS) properties of authentic standards of known hydrocarbon metabolites, a variety of anaerobic metabolites were detected in the plume. This included 3-methylbenzylsuccinic, a characteristic metabolite of anaerobic *m*-xylene biodegradation. Other xylene metabolites were also detected including toluic acid isomers (*o*-, *m*-, and *p*-), *m*-phthalate and carboxybenzaldehydes. These metabolites can form under both aerobic and anaerobic conditions (Gieg et al., 1999b; Elshahed et al., 2001). The detection of 3-methylbenzylsuccinic acid, *m*-toluic acid, and *m*-phthalate is evidence of anaerobic degradation of *m*-xylene. Anaerobic naphthalene metabolites were also detected. A complete list of all metabolites detected can be found in Appendix M.

The finding of a variety of possible anaerobic metabolites in the diesel-impacted groundwater further supports that hydrocarbon contaminants are degrading anaerobically.

6.7.2 AEROBIC DEGRADATION

The lack of measurable biodegradation under anaerobic conditions prompted observation and analysis of aerobic TEH mineralization. Aerobic experiments are often conducted when the groundwater is anaerobic to examine the effect of other limiting factors such as nutrients and temperature (Bregnard et al., 1996; Salanitro et al., 1997). Complete aerobic biodegradation measurements are in Appendix N. The procedure was described in Section 5.3.2.2 and results are shown in Figure 6-18 to Figure 6-21.

6.7.2.1 Trends in Aerobic Biodegradation Data

Unamended aerobic biodegradation of ^{14}C-dodecane at 10°C and 28°C did not exceed 3% mineralization to $^{14}CO_2$. Biodegradation at 28°C was slightly higher than at 10°C (Figure 6-18 and Figure 6-19). Between day 61 and 81 there appeared to be an increase then an immediate decrease in mineralization at both temperatures. This anomaly was also observed in the negative control at 28°C, which indicates possible machine error contributed by the scintillation counter or contamination of the biometers.

These data points were removed from the data set when conducting first order biodegradation analysis.

Aerobic biodegradation in biometers with nutrient amendment (at both temperatures) was significantly higher than in the unamended biometers (Figure 6-18 and Figure 6-19). This clearly indicates that the groundwater at the study site is nutrient limited. Nutrient concentrations in the groundwater were typically low. Nitrate plus nitrite were below the detection limit, phosphate was 0.003 mg/L, and ammonia was 0.01 mg/L. Furthermore, anaerobic microcosms with nutrient amendment showed a decrease in TEH concentrations (Section 6.7.1.4).

Temperature appeared to have an effect on the extent and the rate of dodecane mineralization in the aerobic biometers. At 28°C, the maximum amount of hydrocarbon biodegraded to $^{14}CO_2$ was 36%, with 23% at 10°C. However, biodegradation at 10°C appears to be continuing, so it is possible that mineralization will eventually reach the same level as at 28°C.

Mineralization in the negative controls did not exceed 2% at 28°C and 0.4% at 10°C, indicating that removal of contaminant mass was solely through biodegradation.

6.7.2.2 First Order Biodegradation Rate Data

The first order kinetic model is commonly used to represent biodegradation of an organic compound (Wiedemeier et al., 1996; Chapelle et al., 1996; Wiedemeier et al., 1999). This equation was introduced in Section 4.3.11 as:

$$C_t = C_0 e^{-kt} \qquad [21]$$

In the positive control, the greatest conversion of ^{14}C-dodecane to $^{14}CO_2$ was 42%. Hence, it was assumed that 58% of the radiolabel remained unconverted, and the amount of dodecane remaining at any time (t) was calculated as:

(100%-(100/42 x %^{14}CO$_2$ measured) = %^{14}C-dodecane remaining [35]

This assumption limits the rate constant to an order of magnitude approximation.

Using the converted data, the maximum biodegradation rate was found for the aerobic biometers by plotting ln (%^{14}C-dodecane remaining) versus time (Figure 6-20 and Figure 6-21). The slope of each plot yields the rate constant (k) which are reported in Table 6-13. The half-life was also calculated ($t_{1/2}$ =0.693/k) for each condition. First order rate constants for the nutrient amended samples at both temperatures are much higher than under unamended conditions. The biodegradation rate constants and half-lives were compared to data from Mohn et al. (2000), who examined aerobic degradation of ^{14}C-dodecane in microcosms containing moist soil. The soil microcosms were incubated at various temperatures with adequate nutrients and moisture. The first-order rates from that study are significantly greater than the rates calculated for this study. The half-life for dodecane appears to greatly increase from 7 to 15°C; subsequent increases in temperature do not appear to have such a great effect (Mohn and Stewart, 2000). Comparatively, in this study elevation of temperature decreases the half-life from 0.5 yrs to 0.1 yrs.

Zytner et al. (2001) examined diesel degradation in field experiments and reported aerobic first order rate constants ranging from 0.022 to 0.0043 day^{-1}. These values correspond with the rate constants derived from the nutrient amended biometers.

Table 6-13. Lag Time and First Order Rate Constant for Aerobic Dodecane Mineralization from the Study Site and Mohn et al. (2000)

Sample	Temperature (°C)	k (day^{-1})	k (year^{-1})	$t_{1/2}$ (yr)
	Data from This Study Site			
Unamended	10	0.0002	0.1	9.5
Unamended	28	0.0015	0.5	1.3
Nutrient Amended	10	0.0037	1.4	0.5
Nutrient Amended	28	0.0066	2.4	0.3
	Averaged data from Mohn et al. (2000)			
Average from 3 sites	7	0.3	115.6	0.006
	15	0.9	327.3	0.002
	22	1.5	552.4	0.001
	30	1.6	597.4	0.001

Suarez and Rifai (1999) compared laboratory and field biodegradation rate constants and found results obtained in the laboratory tended to be higher. Laboratory studies tend to maintain ambient conditions for biodegradation, whereas there are often fluctuations in the field. Therefore, the rate constants determined in this study are probably higher than *in-situ* and thus half-lives *in-situ* are potentially longer.

Regression analysis was carried out to examine the fit of the first order model with the aerobic biodegradation data. The validity of application of the first order model to the data was tested by plotting the residuals vs. time (Appendix O). Although the model fits the data, the residuals do not appear to produce a random scatter. Residuals for the unamended microcosms at 10°C are mainly above zero. Residuals for the unamended 28°C and nutrient amended biometers appear to have a cyclic effect, which may indicate influence of time.

6.7.2.3 Lag Time

Lag time is estimated at the 'break-point' when there is a significant increase in recovery of ^{14}C in the form of $^{14}CO_2$. Table 6-14 contains lag times from the aerobic biometers and compares these results with data from Mohn et al. (2000). Lag times for the unamended biometers could not be determined because there was no point where biodegradation appeared to significantly increase. The lag time for the nutrient amended biometers at 28°C was 7 days compared to 15 days at 10°C, which indicates that the optimal growth conditions for the indigenous microorganisms is closer to 28°C than 10°C, or that a component of the population had to become enriched or acclimated before significant activity occurred.

The lag times for the nutrient amended biometers were longer than observed lag times by Mohn et al. (2000). This is probably because the microcosms contained soil, nutrients and sufficient moisture for microorganisms to proliferate, whereas the biometers in this study contained only groundwater.

Table 6-14. Estimated Lag Times from Study Site Aerobic Microcosms and Mohn et al. (2000)

Sample	Temperature (°C)	Lag Time (days)
	Data from Study Site	
Unamended	10	*
Unamended	28	*
Nutrient Amended	10	15
Nutrient Amended	28	7
	Averaged data from Mohn et al. (2000)	
Average from 3 sites	7	6.9
	15	2.8
	22	1.8
	30	1.6

* = No growth phase observed

6.7.2.4 Rate Constant - (Q_{10} and θ)

Q_{10} was defined in Section 3.5.3 as the ratio of the reaction rate at a specific temperature to a rate 10°C lower, and θ was previously described in Section 3.5.2 as a constant that represents the temperature dependence of reaction rates. The response of microbial biodegradation to temperature (as estimated by Q_{10}) is different under unamended and nutrient amended conditions (Table 6-15). The Q_{10} value of 1.38 between 10 and 28°C for the nutrient amended biometers is comparative to the Q_{10} estimate by Mohn et al. (2000) of 1.92 between 15 and 22°C (Table 6-15). Yeung et al. (1997) estimated a Q_{10} value of 2.4 ± 0.3 between 5 and 20°C, which is closer to the Q_{10} value of the unamended biometers.

Reaction rates theoretically double for every 10°C, therefore the expected Q_{10} value would be 2 and θ would equal 1.072. The measured rates are faster than predicted by the theoretical kinetic relationship for the nutrient amended biometers. However, the measured rates appear slower when nutrients are not provided, this further supports the conclusion that there are insufficient nutrients in the groundwater under ambient conditions. With adequate nutrients, microorganisms appear capable of rapid, aerobic mineralization of dodecane at 10°C. This indicates that the indigenous microorganisms are likely psychrotolerant bacteria that are adapted to colder temperatures.

Table 6-15. Temperature Constants, Q_{10} and θ for Aerobic ^{14}C-dodecane Mineralization from the Study Site and Mohn et al. (2000).

Sample	Temperature Range (°C)	Q_{10}	θ
Study Site Samples			
Unamended	10-28	3.06	1.12
Nutrient Amended	10-28	1.38	1.03
Averaged Data from Mohn et al. (2000)			
Average from 3 sites	7-15	3.67	1.14
	15-22	1.92	1.08
	22-30	1.10	1.01

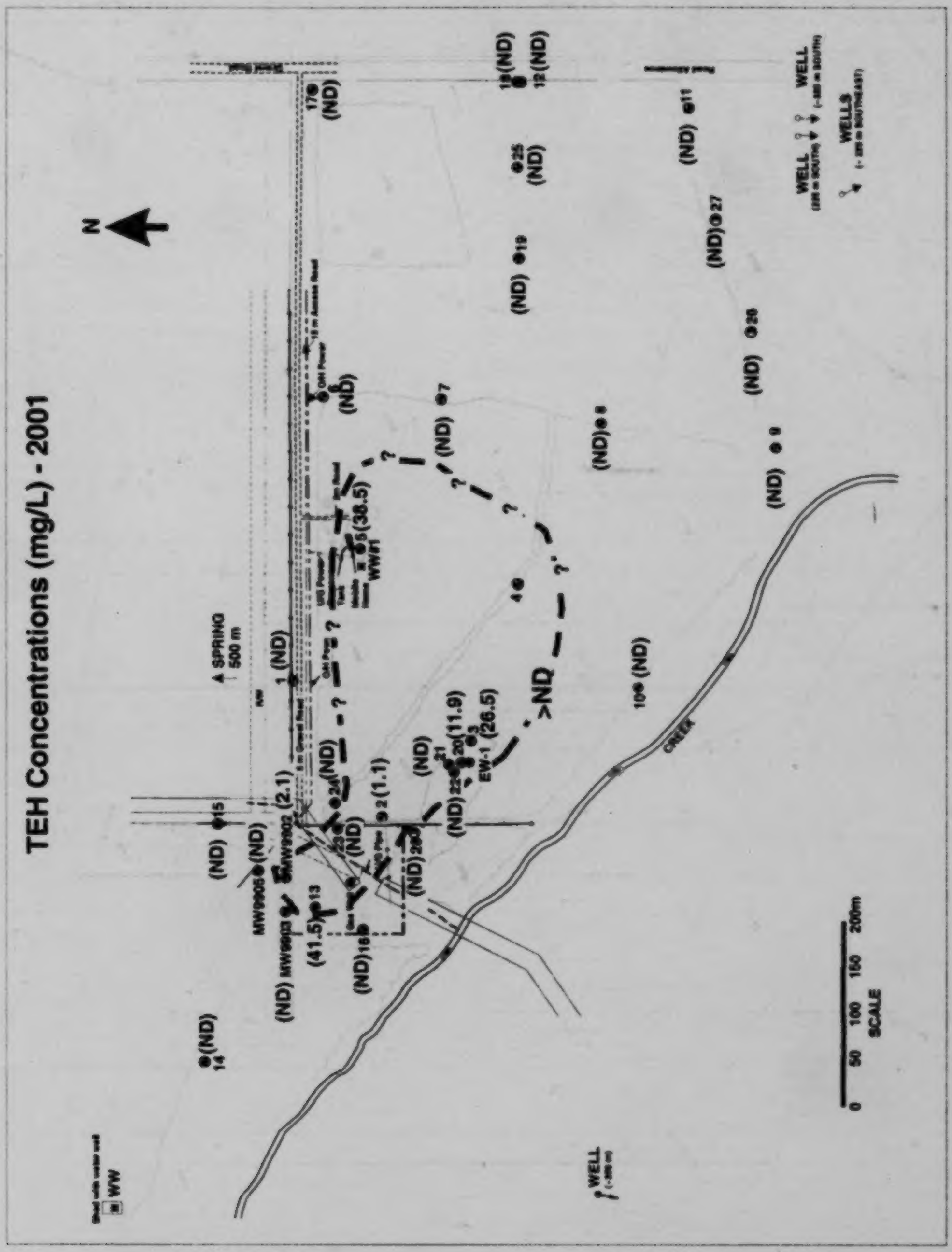

TEH Concentrations (mg/L) - 2001
N
SPRING
500 m
CREEK
>ND
(ND) 15
MW9905 (ND)
MW9902 (2.1)
MW9903
(ND)
(41.5) 13
(ND) 18
14 (ND)
24 (ND)
23 (ND)
(ND) 25
2 (1.1)
(ND)
21
(ND) 22
20 (11.9)
3
EW-1 (26.5)
1 (ND)
4
10 (ND)
5 (38.5)
WWF1
(ND)
(ND) 7
(ND) 8
(ND) 9
(ND) 26
(ND) 27
(ND) 19
25 (ND)
(ND) 11
17 (ND)
12 (ND)
WW
WELL
WELLS
SCALE
0 50 100 150 200m

Figure 6-1. Map of TEH Concentrations (mg/L) in 2000 and 2001. ND = Non Detect

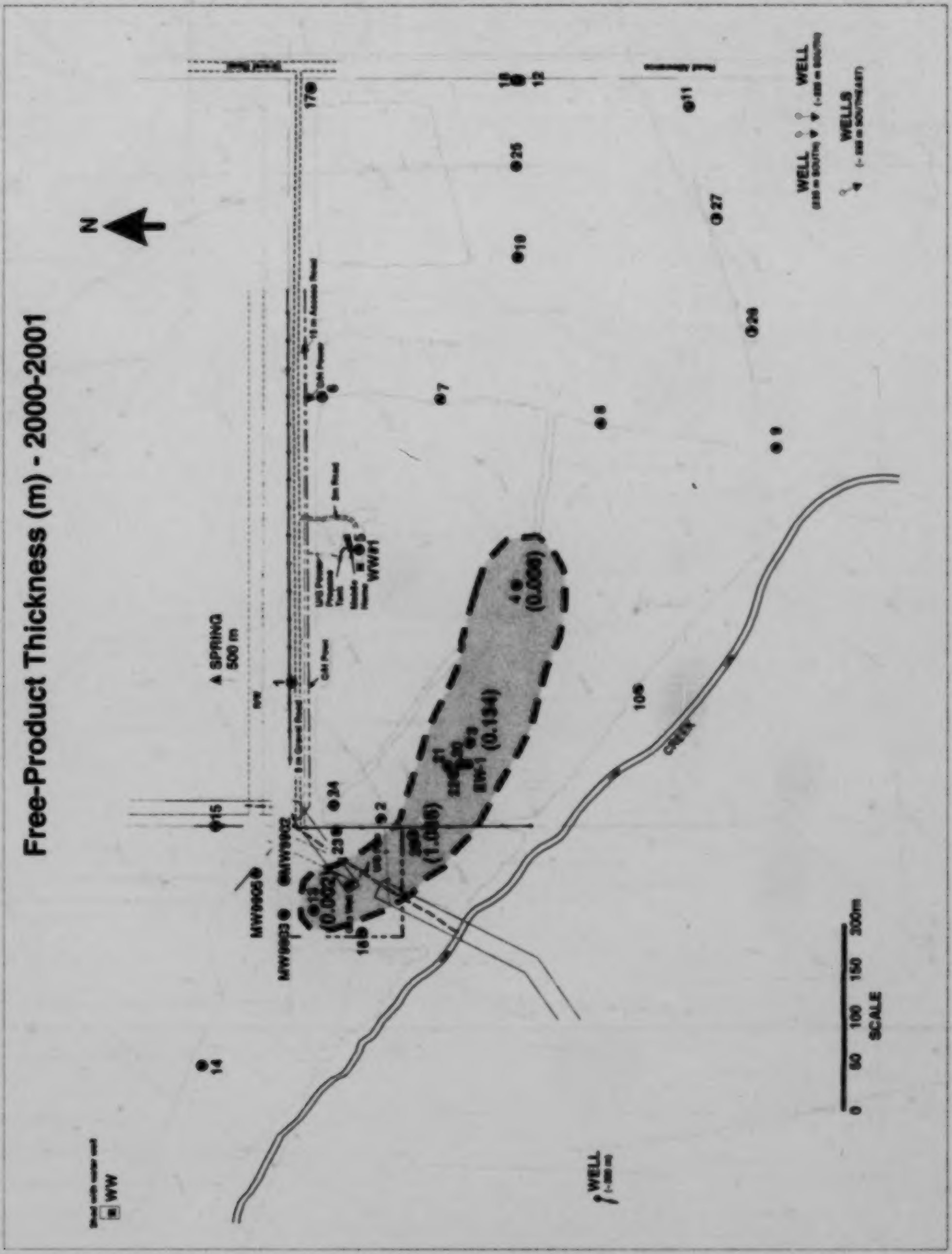

Free-Product Thickness (m) - 2000-2001
N
SPRING
500 m
WW
(1.086)
(0.134)
(0.008)
(0.002)
MW9903
MW9905
SMW9902
SW-1
1
2
4
5
6
7
8
9
10
11
12
14
15
16
17
18
21
22
23
24
25
26
27
105
WW#1
CREEK
WELL
WELLS
SCALE
0
50
100
150
200m

Figure 6-2. Map Showing Lateral Extent and Thickness of Free-Product (m) in the Aquifer between 2000 and 2001

Figure 6-3. Photograph of Free-Product Extracted from Well 3

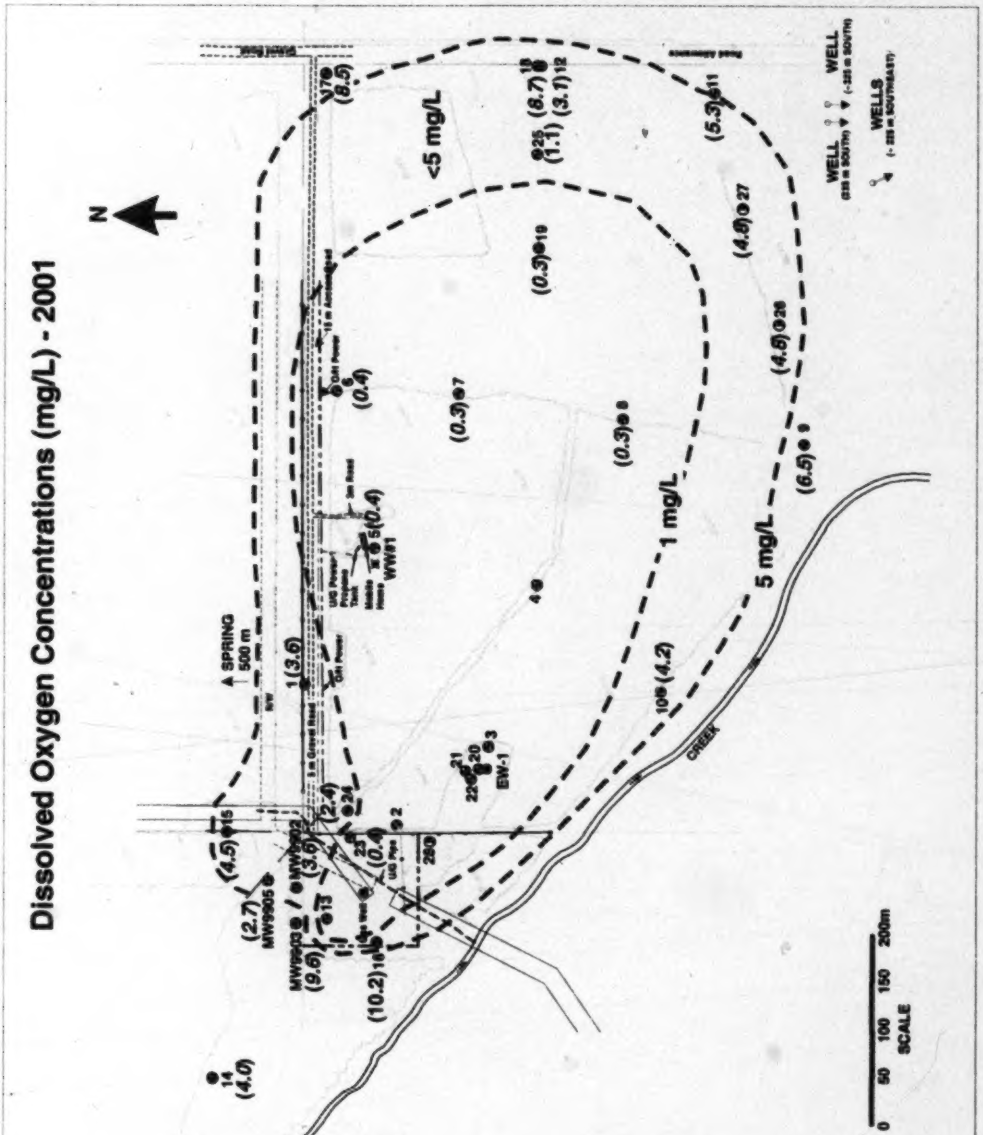
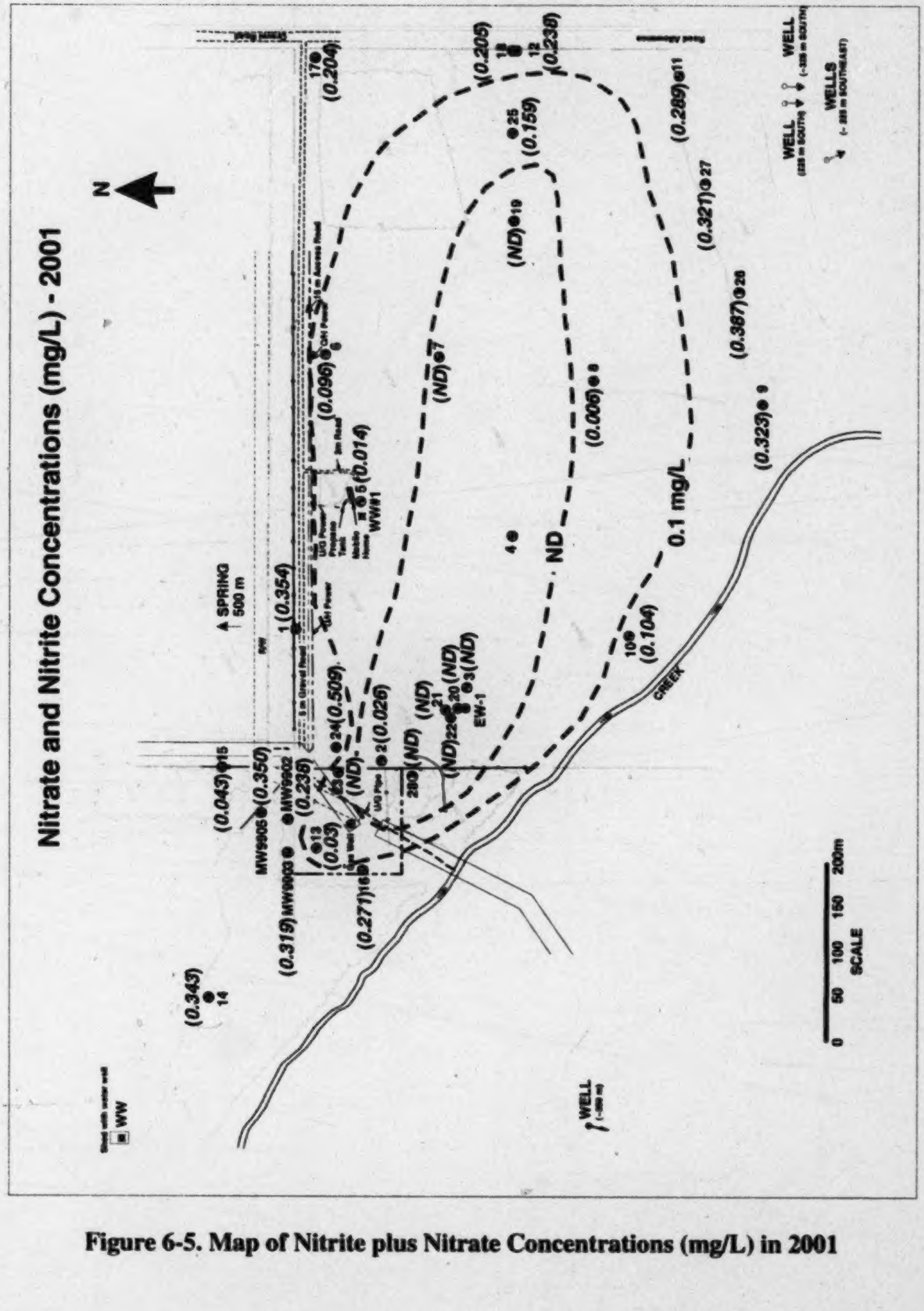

Dissolved Oxygen Concentrations (mg/L) - 2001
N
SPRING
500 m
<5 mg/L
1 mg/L
5 mg/L
CREEK
BW-1
WW1
MW9905
MW9902
MW9903
WW
WELL
WELLS
SCALE
0 50 100 150 200m

Figure 6-4. Map of Dissolved Oxygen Concentrations (mg/L) in 2001



Nitrate and Nitrite Concentrations (mg/L) - 2001
N
SPRING 500 m
0.1 mg/L
ND
CREEK
(0.343) 14
(0.319) MW9903
MW9905 (0.350)
MW9902 (0.236)
(0.043) 15
13 (0.03)
(0.271) 16
24 (0.509)
23 (ND)
2 (0.026)
28 (ND)
(ND) 22
21 (ND)
20 (ND)
3 (ND)
EW-1
1 (0.354)
5 (0.014)
WW#1
4
10 (0.104)
(ND) 7
(0.096) 6
(0.006) 8
(0.323) 9
(0.387) 28
(0.321) 27
(ND) 19
25 (0.159)
18 (0.205)
12 (0.238)
17 (0.204)
(0.289) 11
WELL
WELLS
WW
0 50 100 150 200m
SCALE

Figure 6-5. Map of Nitrite plus Nitrate Concentrations (mg/L) in 2001

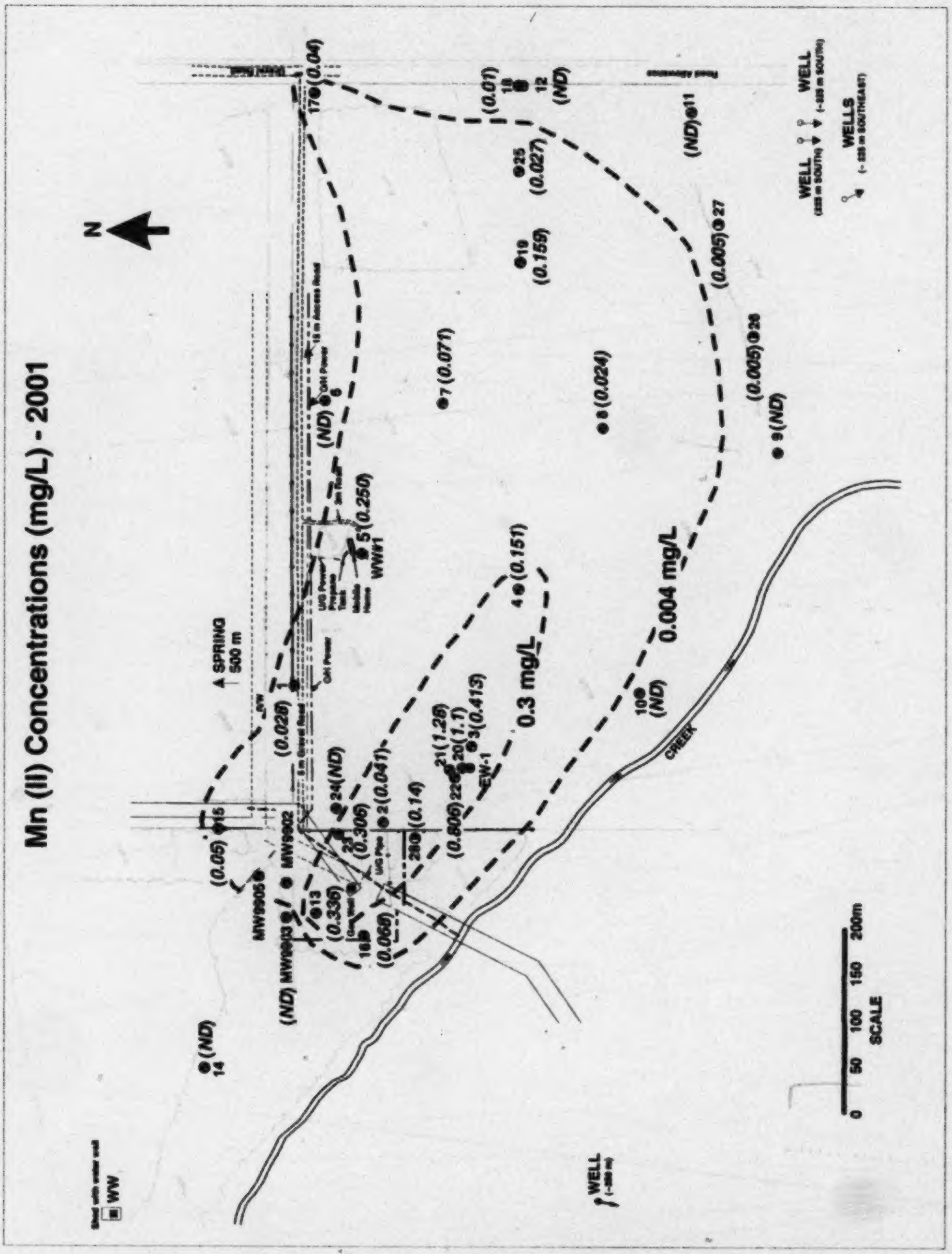

Mn (II) Concentrations (mg/L) - 2001
N
SPRING 500 m
0.3 mg/L
0.004 mg/L
CREEK
14 (ND)
(ND) MW9903
MW9905
(0.05) 15
MW9902
13 (0.336)
18 (0.066)
24 (ND)
23 (0.306)
2 (0.041)
(0.14)
21 (1.28)
20 (1.1)
22 (0.805)
3 (0.413)
EW-1
(0.028)
4 (0.151)
10 (ND)
5 (0.250)
WW#1
(ND) 6
7 (0.071)
8 (0.024)
9 (ND)
(0.005) 26
(0.005) 27
19 (0.159)
25 (0.027)
(ND) 11
16 (0.01)
12 (ND)
17 (0.04)
WELL
WELLS
WW
SCALE
0 50 100 150 200m

Figure 6-6. Map of Manganese (II) Concentrations (mg/L) in 2001

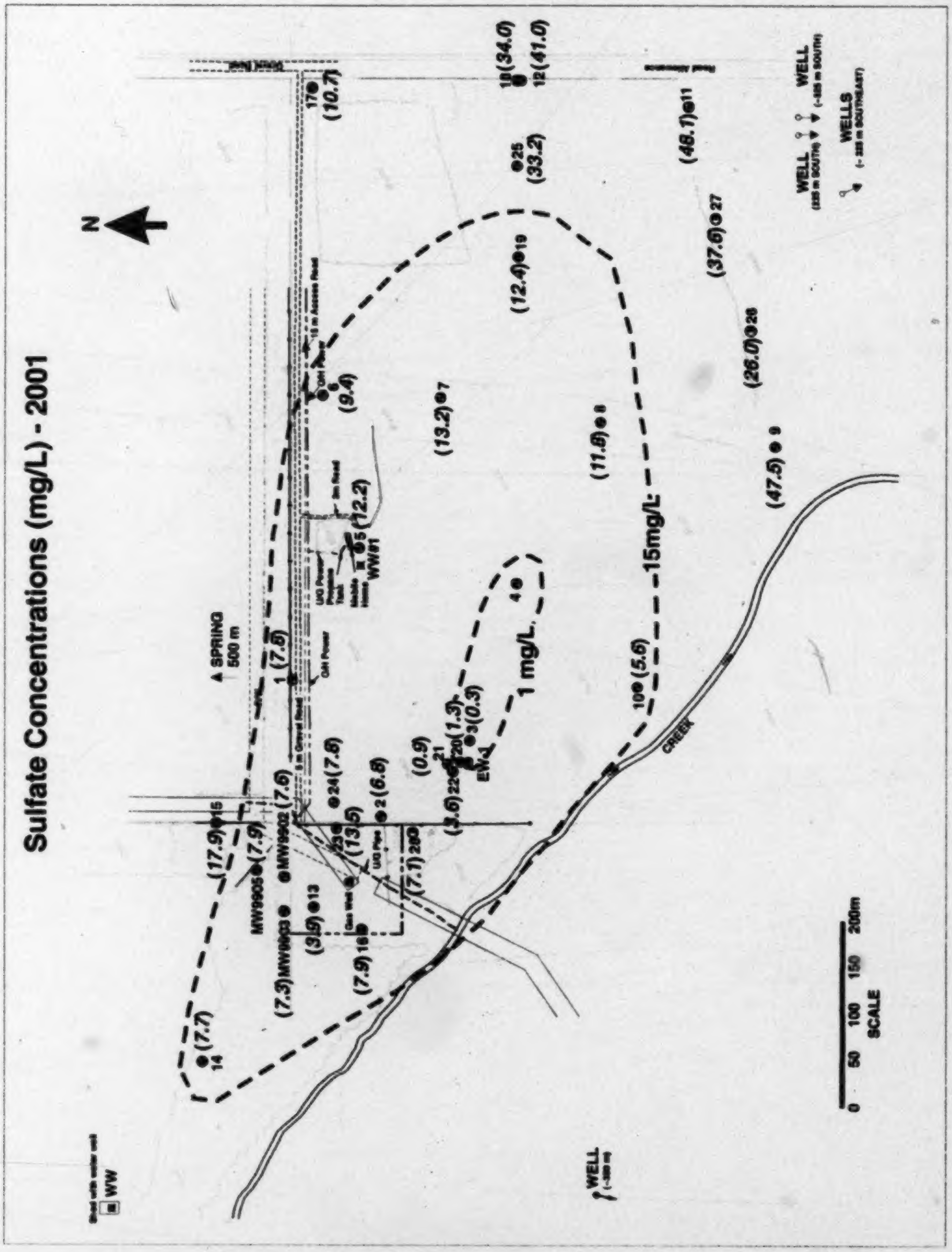

Sulfate Concentrations (mg/L) - 2001
N
SPRING 500 m
WW
WELL
WELL
WELLS
SCALE
0 50 100 150 200m
CREEK
1 mg/L
15mg/L
14 (7.7)
(7.3) MW9903
MW9905 (7.9)
MW9902 (7.6)
(17.9) 15
(3.9) 13
(7.9) 18
23 (13.5)
24 (7.8)
2 (6.8)
(7.1) 28
(0.9) 21
(3.6) 22
20 (1.3)
3 (0.3)
EW-1
1 (7.5)
4
5 (12.2)
WW#1
6 (9.4)
(13.2) 7
(11.8) 8
(47.5) 9
10 (5.6)
(12.4) 19
(26.0) 26
(37.6) 27
25 (33.2)
(48.1) 11
17 (10.7)
16 (34.0)
12 (41.0)

Figure 6-7. Map of Dissolved Sulfate Concentrations (mg/L) in 2001

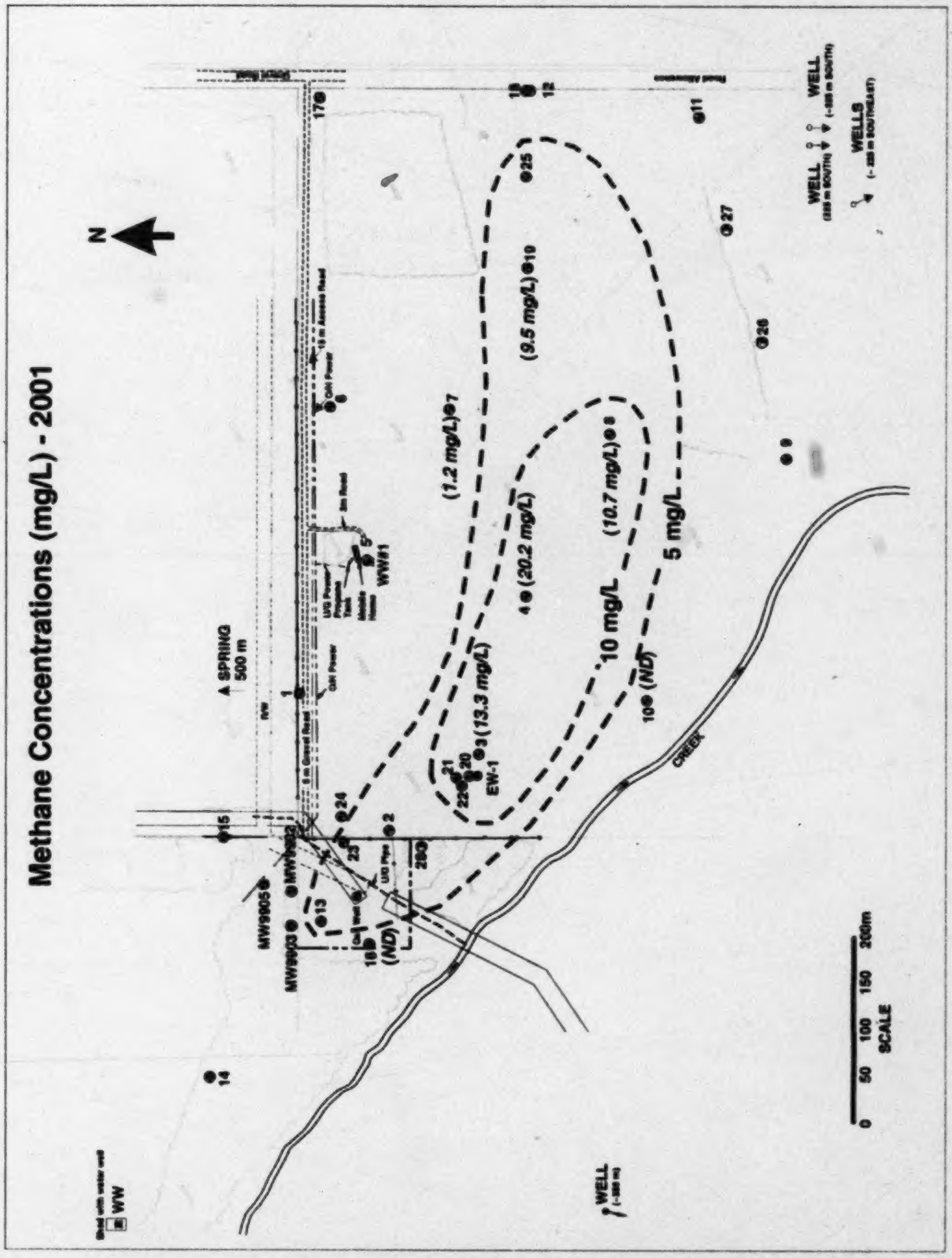
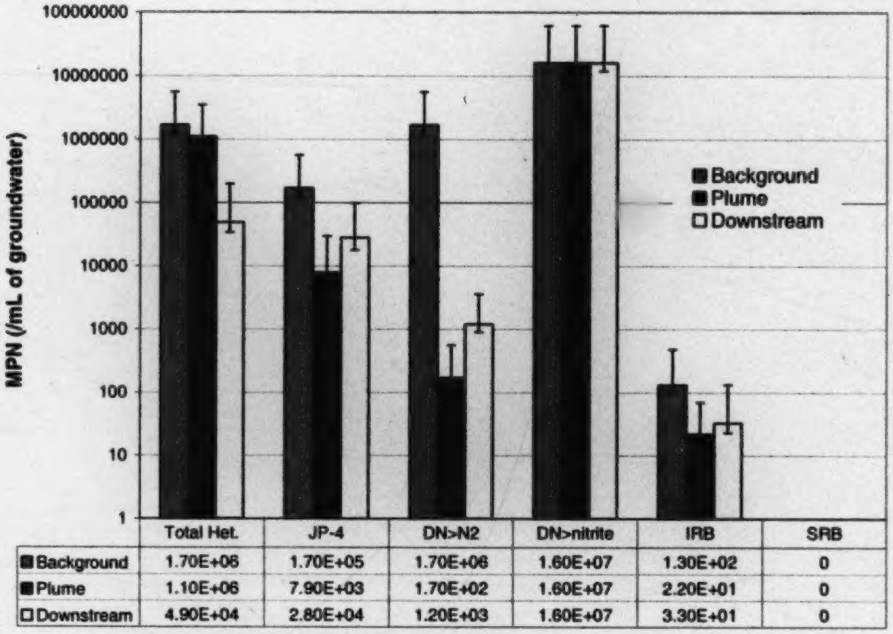

Methane Concentrations (mg/L) - 2001
N
SPRING
500 m
5 mg/L
10 mg/L
(1.2 mg/L)
(9.5 mg/L)
(10.7 mg/L)
(20.2 mg/L)
(13.3 mg/L)
(ND)
(ND)
CREEK
EW-1
WW#1
MW9903
MW9905
MW9902
1
2
3
4
7
8
9
10
11
12
13
14
15
16
17
18
20
21
22
23
24
25
26
27
28
WW
WELL
WELL
WELLS
SCALE
0
50
100
150
200m

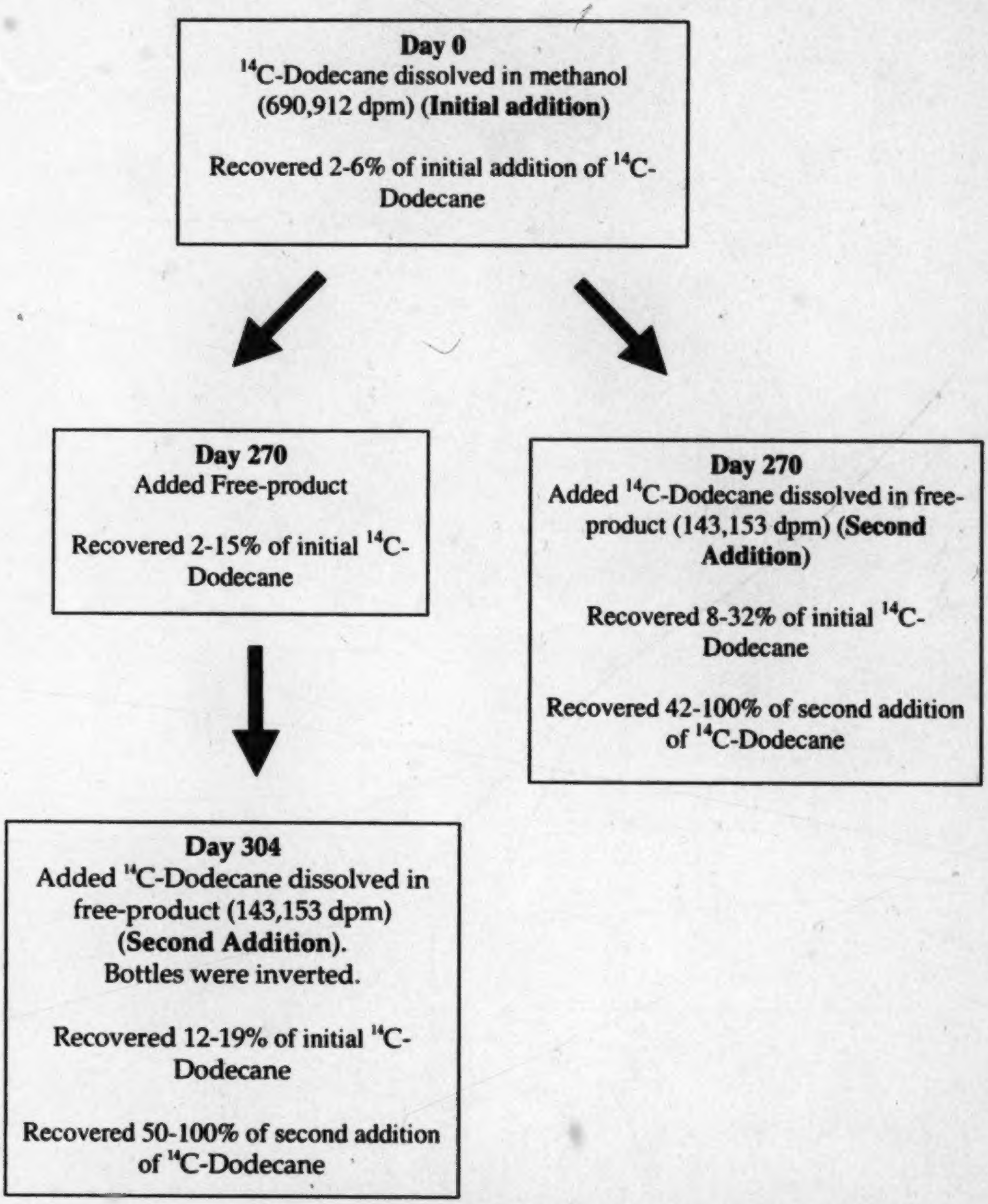
Figure 6-8. Map of Dissolved Methane Concentrations (mg/L) in 2001



	Total Het.	JP-4	DN>N2	DN>nitrite	IRB	SRB
■ Background	1.70E+06	1.70E+05	1.70E+06	1.60E+07	1.30E+02	0
■ Plume	1.10E+06	7.90E+03	1.70E+02	1.60E+07	2.20E+01	0
□ Downstream	4.90E+04	2.80E+04	1.20E+03	1.60E+07	3.30E+01	0

Total Het: Total heterotrophic bacteria
JP-4: JP-4 (jet fuel) degrading bacteria
DN>Nitrate: Denitrifiers that convert nitrate to nitrite
DN>N_2: Denitrifiers that convert nitrate to nitrogen gas
IRB: Iron reducing bacteria
SRB: Sulfate Reducing Bacteria

Figure 6-9. Most Probable Number Results of Groundwater from Study Site after 6 weeks of Incubation at 10°C. Error bars show 95% confidence intervals.



Day 0
^{14}C-Dodecane dissolved in methanol (690,912 dpm) **(Initial addition)**

Recovered 2-6% of initial addition of ^{14}C-Dodecane

Day 270
Added Free-product

Recovered 2-15% of initial ^{14}C-Dodecane

Day 270
Added ^{14}C-Dodecane dissolved in free-product (143,153 dpm) **(Second Addition)**

Recovered 8-32% of initial ^{14}C-Dodecane

Recovered 42-100% of second addition of ^{14}C-Dodecane

Day 304
Added ^{14}C-Dodecane dissolved in free-product (143,153 dpm) **(Second Addition).**
Bottles were inverted.

Recovered 12-19% of initial ^{14}C-Dodecane

Recovered 50-100% of second addition of ^{14}C-Dodecane

Figure 6-10. Flow Chart Outlining Additions and Changes to 125 mL Anaerobic Microcosms

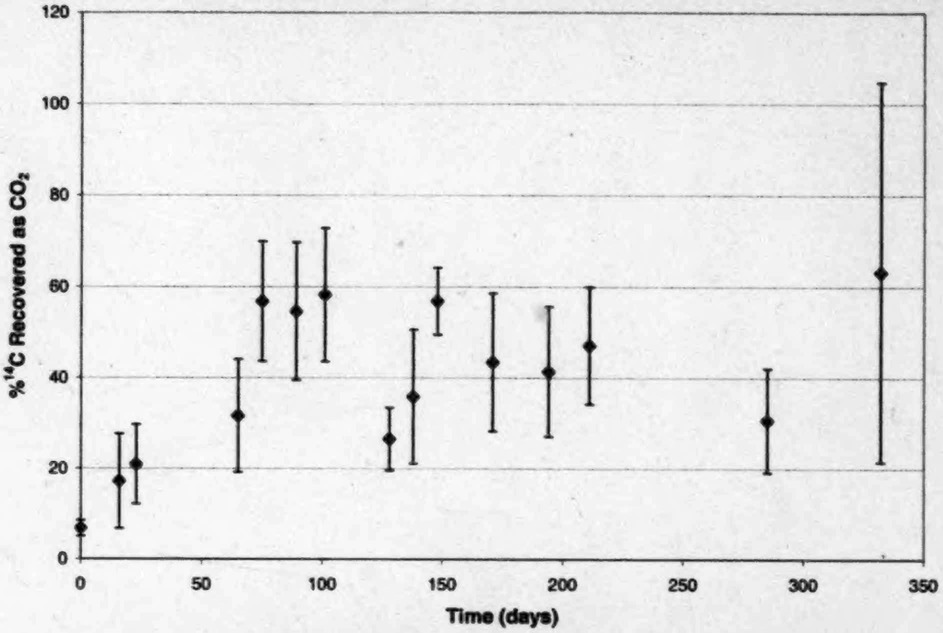


Figure 6-11. Anaerobic Biodegradation of ^{14}C-Acetate in Unamended Microcosms at 10°C. Error bars show one standard deviation (n=3).

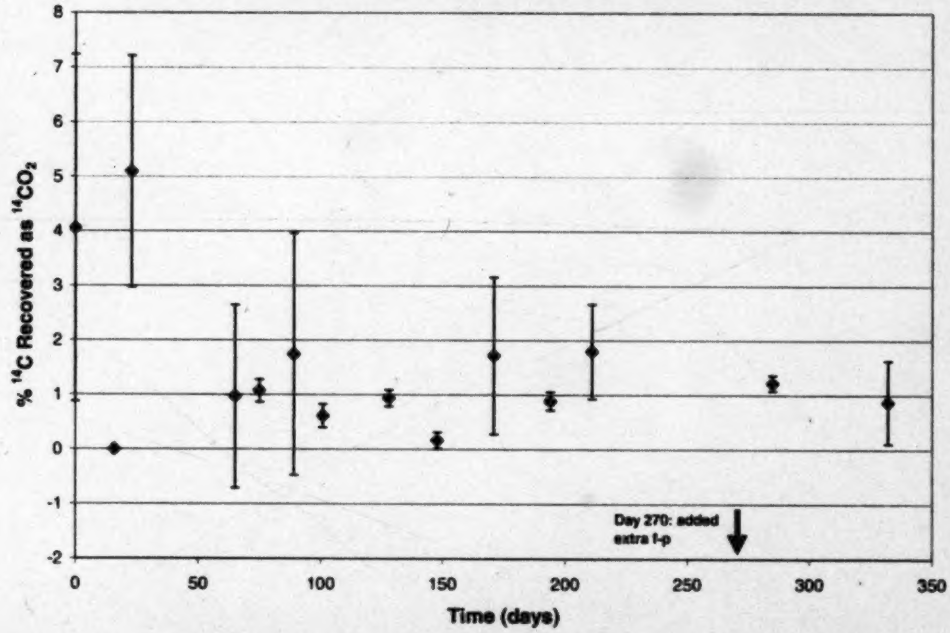


Figure 6-12. Anaerobic Biodegradation of ^{14}C-Toluene in Unamended Microcosms at 10°C. Error bars show one standard deviation (n=3).

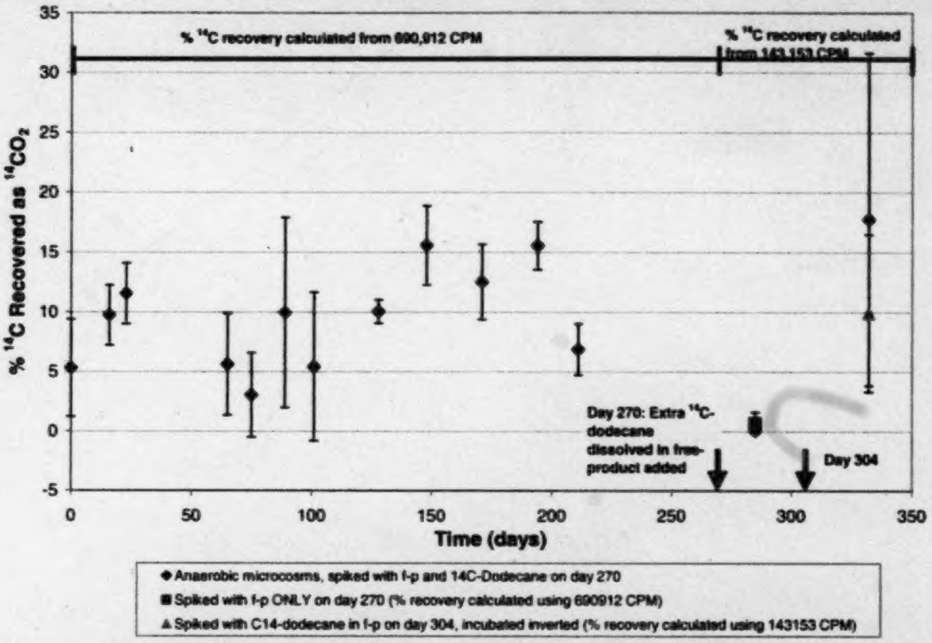


Figure 6-13. Anaerobic Biodegradation of ^{14}C-Dodecane in Nutrient Amended Microcosms at 10°C. Error bars show one standard deviation (n=3).

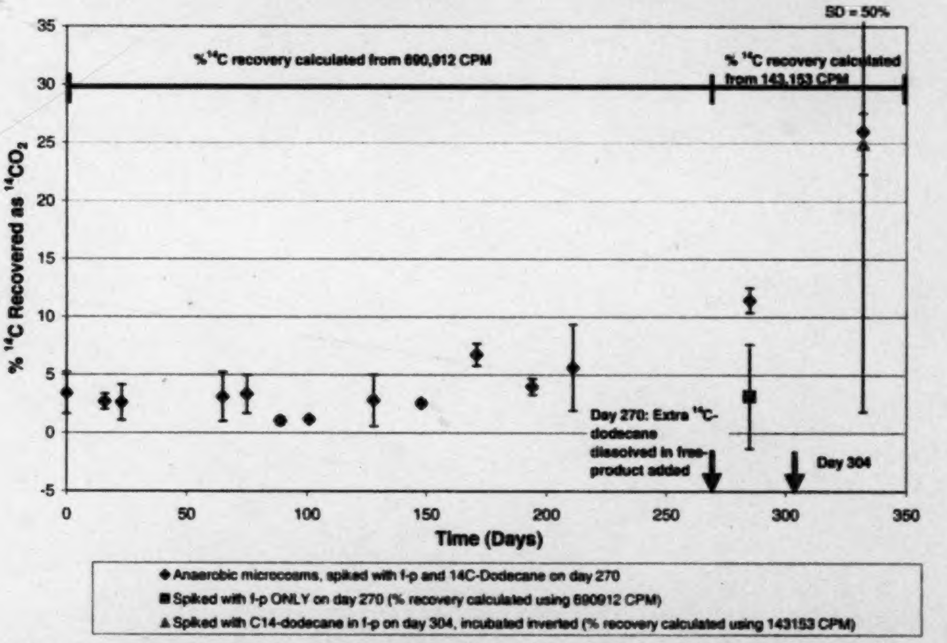


Figure 6-14. Anaerobic Biodegradation of ^{14}C-Dodecane in Nitrate Amended Microcosms at 10°C. Error bars show one standard deviation (n=3).

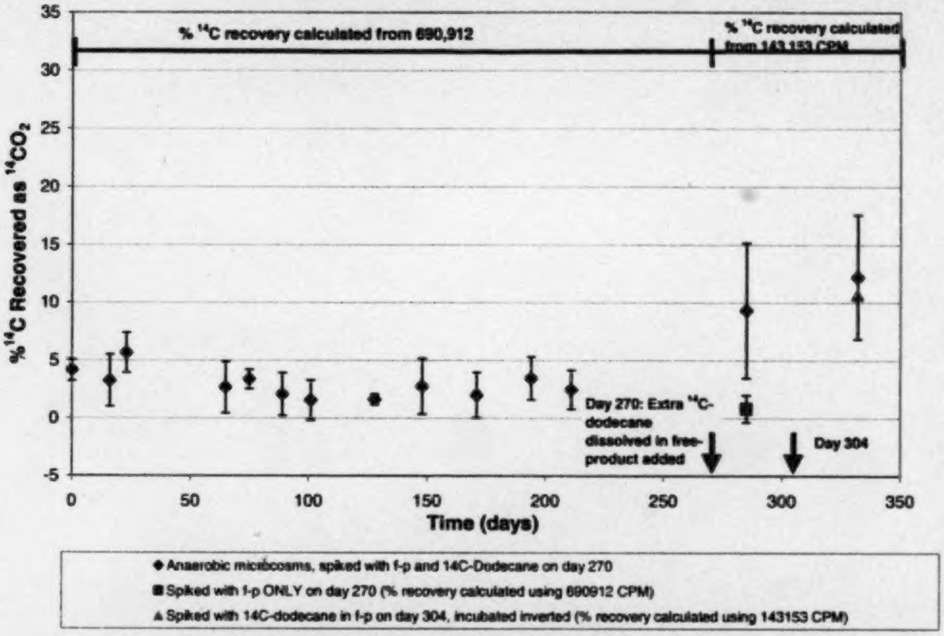


Figure 6-15. Anaerobic Biodegradation of ^{14}C-Dodecane in Unamended Microcosms at 10°C. Error bars show one standard deviation (n=3).

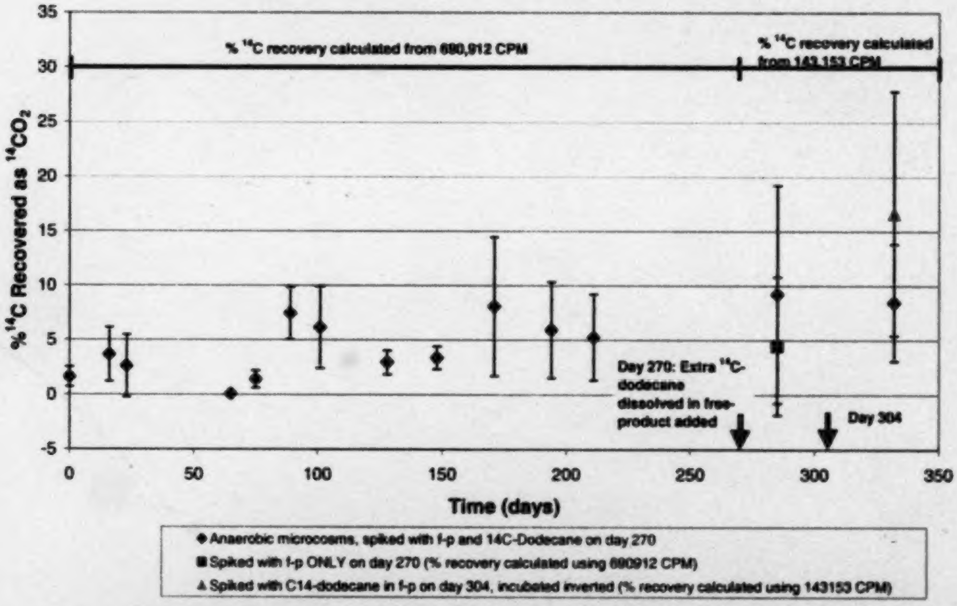


Figure 6-16. Anaerobic Biodegradation of ^{14}C-Dodecane in Sulfate Amended Microcosms at 10°C. Error bars show one standard deviation (n=3).

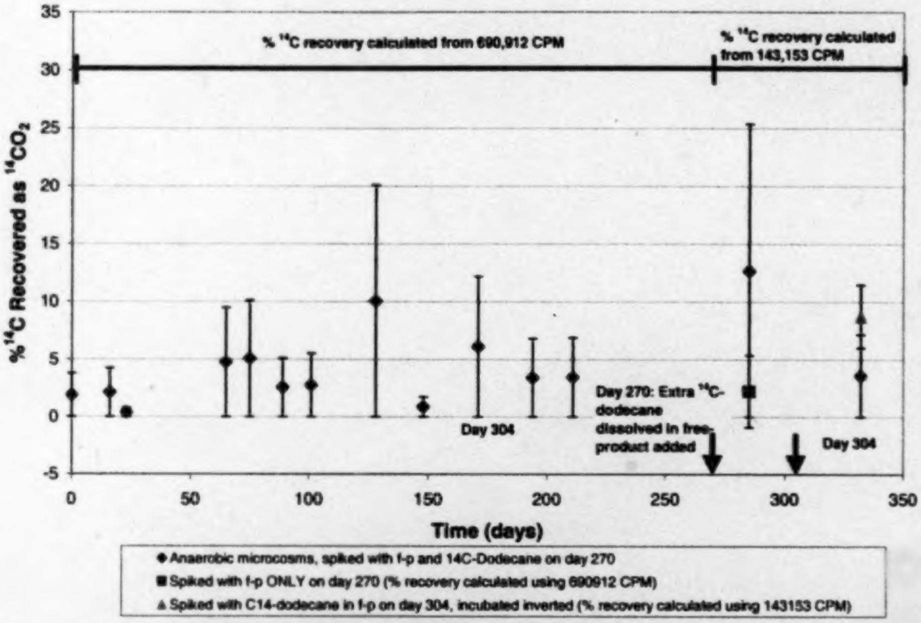


Figure 6-17. Anaerobic Biodegradation of ^{14}C-Dodecane in Unamended Microcosms at 23°C. Error bars show one standard deviation (n=3).

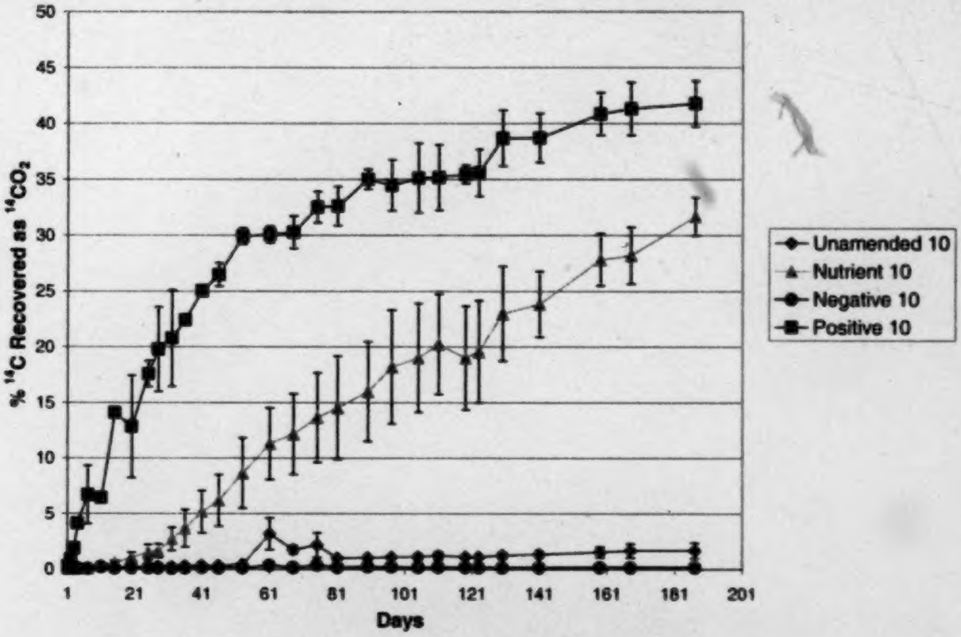


Figure 6-18. Aerobic Microbial Biodegradation of ^{14}C-Dodecane at 10°C. Error bars show one standard deviation (n=3).

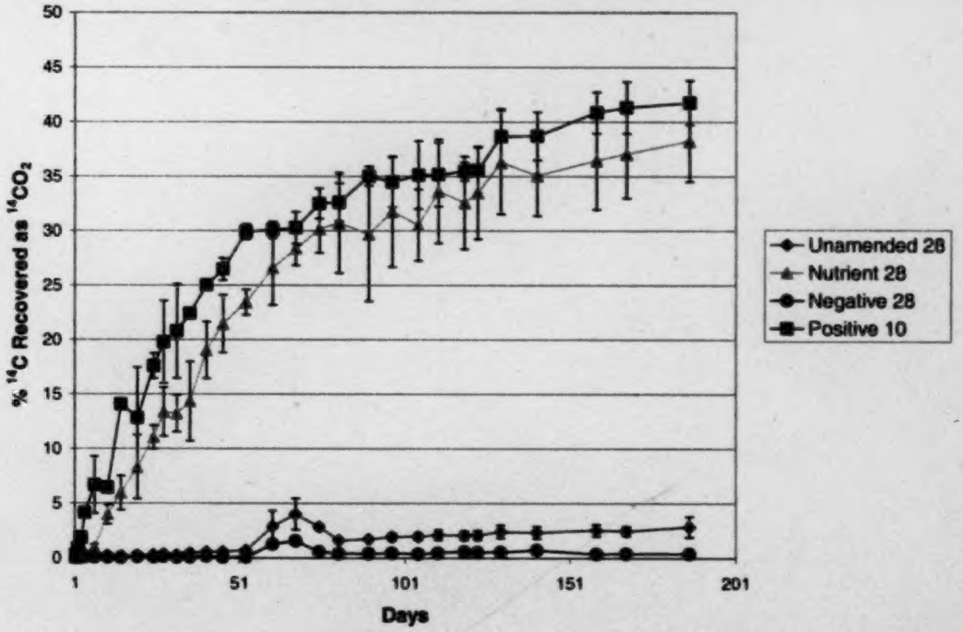


Figure 6-19. Aerobic Microbial Biodegradation of ^{14}C-Dodecane at 28°C. Error bars show one standard deviation (n=3).

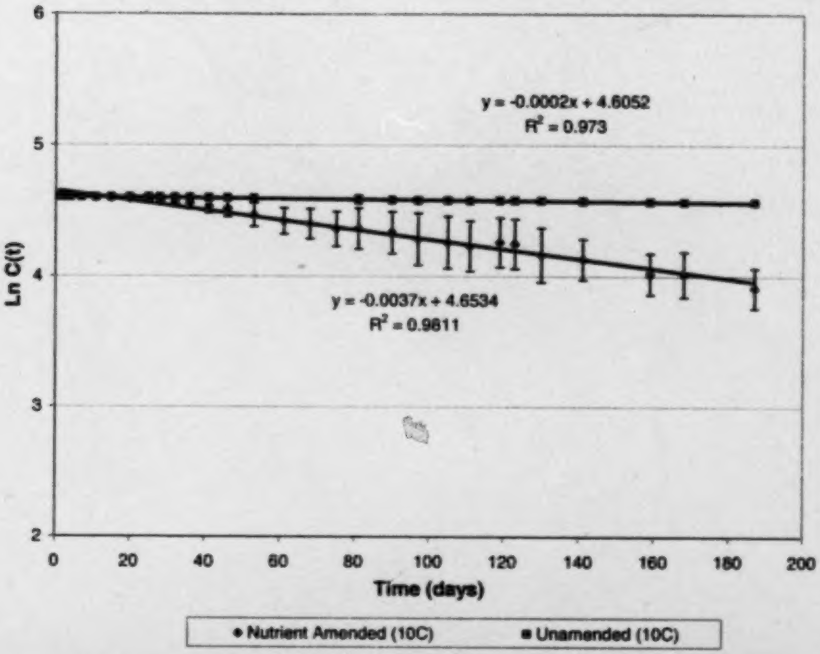


Figure 6-20. First Order Biodegradation Rates for Aerobic Unamended and Nutrient Amended Microcosms at 10°C. C_t = % ^{14}C-dodecane remaining as expressed in equation 35. Error bars show one standard deviation (n=3).

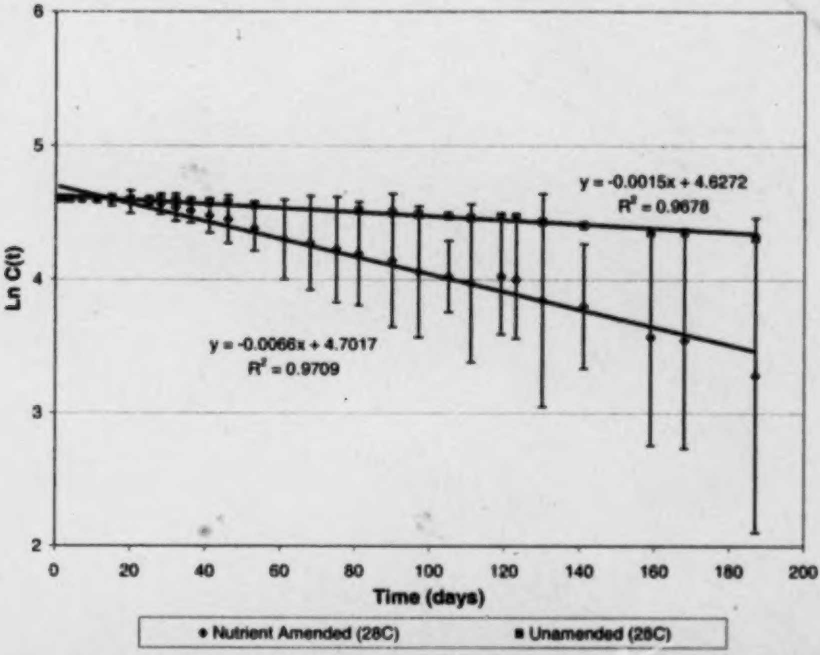


Figure 6-21. First Order Biodegradation Rates for Aerobic Unamended Microcosms at 28°C. C_t = % ^{14}C-dodecane remaining as expressed in equation 35. Error bars show one standard deviation (n=3).

7. LIMITATIONS

7.1 PLUME-A-THON LIMITATIONS

The plume-a-thon database contains detailed information about individual plumes at specific sites. Analysis of the compiled data summarized general trends that appear to influence the effectiveness of NA. However, site heterogeneities that affect the rate of NA may not be adequately captured by the analysis. This limitation especially applies to sites with fractured bedrock, where contamination does not necessarily follow an expected flow path.

Methane analysis was not conducted at any of the site entered into the database. Future site characterization should include sampling for methane in order to get a complete perspective of the contribution of each TEAP to NA.

7.2 CASE STUDY LIMITATIONS

7.2.1 SITE CHARACTERIZATION

Routine groundwater sampling has provided extensive historical data of contaminant concentrations and geochemical indicators, but has not included methane concentrations. Methane analysis should be conducted at all wells to verify if methane production is a result of microbial biodegradation. Additionally, further delineation of TEAP zones could be conducted using dissolved H_2 gas analysis as discussed in Section 3.2.6.

Water sampled with the Waterra and bladder pump produced different results when analyzed for contaminant concentrations. Groundwater samples should be extracted using a bladder pump, because this method minimizes disturbance of the subsurface. Also, the extracted groundwater is more likely to remain anaerobic during sampling, there is less chance of free-product contamination, and there is minimal loss of VOCs by volatilization. Ideally, dedicated bladder pumps should be installed at each well, however this is an expensive endeavour.

7.2.2 MICROBIAL ACTIVITY EXPERIMENTAL PROGRAM

The loss of ^{14}C-dodecane (dissolved in methanol) in the anaerobic microcosms caused inconclusive results. It was difficult to determine from the microcosms whether intrinsic biodegradation is occurring under *in-situ* conditions in the subsurface. Analysis of the fate of the missing dodecane radiolabel may provide future insight for other studies to prevent such loss.

Addition of free-product to laboratory microcosms may be important. Supplementary hydrocarbon was not initially added because the toxicity effect was unknown. It would be advantageous to conduct a toxicity assay to determine the effect of contaminant concentration on indigenous microorganisms.

7.2.3 FIRST ORDER BIODEGRADATION MODEL

There are certain limits in applying the first order model to intrinsic biodegradation reactions. There are a number of simplifying assumptions with respect to site-specific conditions for biodegradation at fuel-contaminated sites (Odermatt, 1997),

a) Degradation rates do not vary with position (*i.e.* depth) in the subsurface or over time;

b) Degradation rates are constant and do not account for changes in the microbial population in time and space;

c) The influence of contaminant loading, toxicity and the soil environment are ignored; and

d) The process is instantaneous and 100% effective at all times wherever contamination occurs.

These assumptions are unlikely to meet conditions for biologically mediated degradation reactions. However, the aerobic biodegradation data in this study have a reasonable fit with the first-order model. Furthermore, the majority of studies model biodegradation using the first-order model, thus use of the first-order model in this study allows direct comparison to other biodegradation studies.

8. CONCLUSIONS

8.1 PLUME-A-THON

The CORONA plume-a-thon database indicates that although NA occurs at many upstream sites, no apparent 'winning' conditions were identified. The use of NA as a remediation option must be assessed on a site-specific basis. The data suggest that 'losing' conditions may be associated with high concentrations of co-contaminants, however, further detailed assessment is required.

The majority of PHC sites (71%) have shrinking or stabilized plumes. This is an indication that natural attenuation is active at upstream facilities. Inorganic and non-PHC plume analysis showed a lower percentage of shrinking plumes and a higher percentage of growing plumes. Furthermore, plume lengths tended to be longer for inorganic and non-PHC plumes compared to PHC plumes.

8.2 CASE-STUDY

The program of investigation was constructed to evaluate the extent of natural attenuation at the study site. Historical evidence indicates that the plume is stable but there were some increases in TEH concentration within the plume at a few monitoring wells. This is believed to have been to free-product contamination during sampling. The plume does not appear to have migrated further downstream during the monitoring period. These results must be approached with caution because the groundwater table is in fractured bedrock. There is the possibility of contaminants migrating through the fractures and not being detected at monitoring wells.

Examination of geochemical and hydrogeological data suggests the groundwater within the plume area and further downstream (well 8) is highly anaerobic. This area has dissolved oxygen concentrations less than 0.5 mg/L. For aerobic respiration to occur oxygen must be present at concentrations greater than 0.5 mg/L (Wiedemeier et al., 1999). This is further supported by decreases in nitrate concentration, increases in

Mn(IV) concentrations, a clear indication of sulfate depletion, and high methane concentrations in the plume. Methanogenesis appears to be an active TEAP within the plume.

The BARTs™ and MPNs confirm the presence of anaerobic bacteria that are capable of degrading diesel contamination. Anaerobic bacteria such as sulfate reducers are active in the plume and absent outside the area of contamination. The population of denitrifiers is higher downstream and upstream than in the plume. This suggests that sulfate is an active TEAP in the contaminated area.

Nutrient addition appeared to significantly improve anaerobic biodegradation. TEH concentrations decreased in the nutrient (45 to 27.1 mg/L) and nitrate amended (45 to 31.7 mg/L) 1-L microcosms. Nitrate concentrations in both sets of experiments also decreased over time. This observation was also supported by nitrate reduction in 125-mL anaerobic microcosms.

TEH biodegradation under unamended conditions in the laboratory did not appear to be effective. There were no decreases in TEH concentration in the 1-L unamended anaerobic microcosms at 10°C. Furthermore, unamended aerobic biometers showed low rates of biodegradation.

Measurement of the anaerobic biodegradation was inconclusive due to the loss of dodecane radiolabel. The radiolabel may have co-evaporated during addition, sorbed to the stoppers, or may not have been bioavailable due to the lack of free-phase hydrocarbon. However, microbial metabolite analysis from samples directly from the study site confirmed that anaerobic degradation of diesel is occurring.

First order biodegradation rates were derived for the study site under aerobic conditions. Biodegradation was extremely low for unamended groundwater at 10°C and 20°C. Nutrient amendment appeared to greatly enhance biodegradation rates at both temperatures, indicating that the groundwater is nutrient deficient. The Q_{10} value was 1.38 between 10 and 28°C in the nutrient amended biometers, and 3.06 in the unamended biometers. This provides further evidence that there are insufficient nutrients in the groundwater under ambient conditions. However, with adequate nutrients, microorganisms appear capable of rapid, aerobic mineralization of dodecane at 10°C.

8.3 FUTURE WORK

Future plume-a-thon research will include expanding the database to include other types of contaminants such as inorganics and process chemicals. Comparisons in plume behaviour will allow a more complete interpretation of the effects of co-contaminants on natural attenuation. There also should be a more detailed evaluation of well-documented sites, to provide data that would support NA.

The information obtained from this study can be used to determine the steps that are required to clean up the study site. The historical and current geochemical and contaminant data can be used to model the study site. Models can be used to predict migration and degradation of the contaminant plume in time and space. Modelling would provide information on the fate of contaminants and whether natural attenuation is occurring via physio-chemical or biological processes. There are a number of analytical and numerical models that can be used. Wiedemeier et al. (1999) should be consulted when selecting an appropriate model.

An investigation of technologies to effectively remove the free-product floating atop the water table would aid in removing the continuous source of contamination. However, it is likely impossible to effectively remove LNAPL within the fractured bedrock.

The EBC at the study site does not appear to be high enough to mineralize the maximum concentration of TEH within the plume at well 3. However, as the contaminants are diluted and carried downstream, the EBC may be adequate to biodegrade contaminants at the plume edge (well 7 and 8). Future work at the study site should include microbial analysis of groundwater from downstream wells to assess whether there is sufficient microbial capacity to effectively remove contaminants.

Anaerobic biodegradation should be reassessed, especially with nutrient additions. It would also be beneficial to further assess the effects of nutrient addition to the groundwater *in-situ*. This would be achieved by setting up a pilot project where controlled amounts of nutrients (below CDWG) are added to the plume. Monitoring will determine whether nutrient amendment will enhance bioremediation *in-situ*.

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APPENDIX A

ROUTINE GROUNDWATER ANALYSES (KOMEX INTERNATIONAL LTD., 2001)

DETAILED CHEMICAL ANALYSIS OF GROUNDWATER FROM WELLS 3 AND 8

Table A-1

Monitoring Well Installation Details, Datum/Groundwater Surface Elevations And Hydraulic Conductivities

Monitoring Station	Ground Elevation (masl)	Stick-Up PVC Pipe (m)	Datum Elevation (top of PVC casing) (masl)	Depth of MW (below ground) (m)	Depth Interval of Sand (below ground) (m)	Date (d-m-y)	Depth To Water Below Datum (m)	Depth To Product Below Datum (m)	Apparent Condensate Thickness (m)	Groundwater Surface Elevation (masl)	Hydraulic Conductivity (m/s)	Lithology
1	1,117.78	0.62	1,118.41	42.15	35.30 - 42.15	24-Jul-96	33.71	---	---	1,084.70	> 1E-04	Sandstone
						31-Jul-96	33.69	---	---	1,084.72		
						29-Aug-96	33.73	---	---	1,084.68		
						19-Sep-96	33.82	---	---	1,084.59		
						5-Nov-96	34.08	---	---	1,084.33		
						6-Dec-96	34.25	---	---	1,084.16		
						11-Feb-97	34.49	---	---	1,083.92		
						2-Apr-97	34.52	---	---	1,083.89		
						30-Apr-97	33.97	---	---	1,084.44		
						27-May-97	33.82	---	---	1,084.59		
						3-Jun-97	33.60	---	---	1,084.81		
						4-Jul-97	33.51	---	---	1,084.90		
						26-Aug-97	34.16	---	---	1,084.25		
						19-Nov-97	33.71	---	---	1,084.70		
						22-May-98	34.43	---	---	1,083.98		
						11-May-99	34.45	---	---	1,083.96		
						26-Jan-00	33.87	---	---	1,084.54		
						18-Sep-00	33.18	---	---	1,085.23		
						25-Apr-01	34.01	---	---	1,084.40		
2	1,115.82	0.63	1,116.45	38.30	31.40 - 38.30	24-Jul-96	31.34	---	---	1,085.11	> 1E-04	Sandstone
						31-Jul-96	31.21	---	---	1,085.24		
						29-Aug-96	31.24	---	---	1,085.21		
						19-Sep-96	31.34	---	---	1,085.11		
						5-Nov-96	31.58	---	---	1,084.87		
						6-Dec-96	31.73	---	---	1,084.72		
						11-Feb-97	31.99	---	---	1,084.47		
						2-Apr-97	32.00	---	---	1,084.45		
						30-Apr-97	31.49	---	---	1,084.97		
						3-Jun-97	31.16	---	---	1,085.30		
						4-Jul-97	31.05	---	---	1,085.40		
						26-Aug-97	31.84	---	---	1,084.61		
						19-Nov-97	31.19	---	---	1,085.27		
						22-May-98	31.92	---	---	1,084.54		
						13-Jul-98	30.05	---	---	1,086.41		
						21-Aug-98	30.36	---	---	1,086.10		
						11-May-99	31.95	---	---	1,084.50		
						26-Jan-00	31.37	---	---	1,085.08		
						19-Sep-00	30.71	---	---	1,085.74		
						25-Apr-01	31.55	---	---	1,084.91		

Table A-1

Monitoring Well Installation Details, Datum/Groundwater Surface Elevations And Hydraulic Conductivities

Monitoring Station	Ground Elevation	Stick-Up PVC Pipe	Datum Elevation (top of PVC casing)	Depth of MW (below ground)	Depth Interval of Sand (below ground)	Date	Depth To Water Below Datum	Depth To Product Below Datum	Apparent Condensate Thickness	Groundwater Surface Elevation	Hydraulic Conductivity	Lithology
	(masl)	*(m)*	*(masl)*	*(m)*	*(m)*	*(d-m-y)*	*(m)*	*(m)*	*(m)*	*(masl)*	*(m/s)*	
3	1,115.35	0.69	1,116.03	36.20	27.70 - 36.20	24-Jul-96	31.02	---	---	1,085.02	> 1E-04	Sandstone
						31-Jul-96	30.97	---	---	1,085.06		
						29-Aug-96	30.98	---	---	1,085.05		
						19-Sep-96	31.07	---	---	1,084.96		
						5-Nov-96	31.34	---	---	1,084.69		
						6-Dec-96	31.50	---	---	1,084.53		
						11-Feb-97	31.99	31.74	0.250	1,084.24		
						2-Apr-97	31.81	---	---	1,084.22		
						30-Apr-97	31.34	---	---	1,084.69		
						3-Jun-97	31.03	---	---	1,085.00		
						4-Jul-97	30.84	---	---	1,085.19		
						26-Aug-97	31.87	---	---	1,084.16		
						19-Nov-97	31.11	30.86	0.255	1,085.12		
						22-May-98	31.94	31.68	0.265	1,084.31		
						13-Jul-98	30.13	29.93	0.200	1,086.06		
						20-Jul-98	30.22	30.02	0.198	1,085.98		
						6-Aug-98	30.03	30.02	0.011	1,086.02		
						5-Nov-98	30.88	30.66	0.220	1,085.33		
						18-Nov-98	31.09	30.79	0.300	1,085.18		
						24-Nov-98	31.10	30.84	0.260	1,085.14		
						13-Jan-99	31.53	31.25	0.274	1,084.72		
						29-Jan-99	31.62	31.34	0.277	1,084.64		
						30-Jan-99	31.41	31.38	0.039	1,084.65		
						11-Feb-99	31.54	31.44	0.103	1,084.58		
						3-Mar-99	31.67	31.53	0.131	1,084.47		
						11-May-99	31.93	31.71	0.218	1,084.28		
3 (Post-Purge)						11-May-99	31.79	31.73	0.058	1,084.29		
						28-Jan-00	31.38	31.11	0.270	1,084.87		
						19-Sep-00	30.72	30.33	0.390	1,085.63		
						25-Apr-01	31.45	31.32	0.134	1,084.69		

Table A-1

Monitoring Well Installation Details, Datum/Groundwater Surface Elevations And Hydraulic Conductivities

Monitoring Station	Ground Elevation	Stick-Up PVC Pipe	Datum Elevation (top of PVC casing)	Depth of MW (below ground)	Depth Interval of Sand (below ground)	Date	Depth To Water Below Datum	Depth To Product Below Datum	Apparent Condensate Thickness	Groundwater Surface Elevation	Hydraulic Conductivity	Lithology
	(masl)	*(m)*	*(masl)*	*(m)*	*(m)*	*(d-m-y)*	*(m)*	*(m)*	*(m)*	*(masl)*	*(m/s)*	
4	1,118.31	0.72	1,119.03	39.00	30.50 - 39.00	24-Jul-96	34.24	---	---	1,084.79	> 1E-04	Sandstone
						31-Jul-96	34.20	---	---	1,084.83		
						29-Aug-96	34.20	---	---	1,084.83		
						19-Sep-96	34.29	---	---	1,084.74		
						5-Nov-96	34.56	---	---	1,084.47		
						6-Dec-96	34.73	---	---	1,084.30		
						11-Feb-97	35.00	---	---	1,084.03		
						2-Apr-97	35.05	---	---	1,083.98		
						30-Apr-97	34.67	34.60	0.075	1,084.42		
						3-Jun-97	34.34	34.28	0.060	1,084.74		
						4-Jul-97	34.14	34.06	0.080	1,084.95		
						26-Aug-97	35.18	---	---	1,083.85		
						19-Nov-97	34.18	34.09	0.090	1,084.92		
						22-May-98	35.07	34.94	0.125	1,084.06		
						20-Jul-98	33.36	33.35	0.009	1,085.68		
						6-Aug-98	33.29	33.26	0.033	1,085.77		
						13-Jan-99	34.59	34.50	0.096	1,084.51		
						29-Jan-99	34.68	34.59	0.098	1,084.42		
						30-Jan-99	34.61	34.60	0.010	1,084.43		
						11-Feb-99	34.75	34.66	0.081	1,084.35		
						3-Mar-99	34.86	34.77	0.089	1,084.24		
						11-May-99	35.03	34.97	0.058	1,084.05		
						28-Jan-00	34.44	34.35	0.090	1,084.66		
						19-Sep-00	33.59	---	---	1,085.44		
						25-Apr-01	34.60	34.59	0.008	1,084.44		
5	1,119.28	-1.78	1,117.50	38.60	30.00 - 38.60	19-Jul-96	33.23	---	---	1,084.27	> 1E-04	Sandstone
						24-Jul-96	32.80	---	---	1,084.70		
						29-Aug-96	33.01	---	---	1,084.49		
						19-Sep-96	32.90	---	---	1,084.60		
						5-Nov-96	33.36	---	---	1,084.14		
						6-Dec-96	N/M	---	---	N/M		
						11-Feb-97	N/M	---	---	N/M		
						2-Apr-97	N/M	---	---	N/M		
						30-Apr-97	33.31	---	---	1,084.19		
						3-Jun-97	32.95	---	---	1,084.55		
						4-Jul-97	32.84	---	---	1,084.66		
						26-Aug-97	N/M	---	---	N/M		
						19-Nov-97	32.97	---	---	1,084.53		
						22-May-98	33.71	---	---	1,083.79		
						11-May-99	33.74	---	---	1,083.76		
						27-Jan-00	33.16	---	---	1,084.34		
						18-Sep-00	32.48	---	---	1,085.02		
						25-Apr-01	33.30	---	---	1,084.19		

Table A-1

Monitoring Well Installation Details, Datum/Groundwater Surface Elevations And Hydraulic Conductivities

Monitoring Station	Ground Elevation (masl)	Stick-Up PVC Pipe (m)	Datum Elevation (top of PVC casing) (masl)	Depth of MW (below ground) (m)	Depth Interval of Sand (below ground) (m)	Date (d-m-y)	Depth To Water Below Datum (m)	Depth To Product Below Datum (m)	Apparent Condensate Thickness (m)	Groundwater Surface Elevation (masl)	Hydraulic Conductivity (m/s)	Lithology
6	1,119.36	0.64	1,120.00	42.31	36.00 - 42.31	5-Nov-96	38.35	---	---	1,081.65	> 1E-04	Sandstone
						6-Dec-96	38.47	---	---	1,081.54		
						11-Feb-97	38.67	---	---	1,081.33		
						2-Apr-97	38.72	---	---	1,081.28		
						30-Apr-97	38.22	---	---	1,081.79		
						3-Jun-97	37.86	---	---	1,082.15		
						4-Jul-97	37.85	---	---	1,082.15		
						26-Aug-97	39.41	---	---	1,080.59		
						19-Nov-97	38.09	---	---	1,081.92		
						21-May-98	38.58	---	---	1,081.42		
						11-May-99	38.58	---	---	1,081.42		
						26-Jan-00	38.11	---	---	1,081.89		
						18-Sep-00	37.65	---	---	1,082.35		
						25-Apr-01	38.10	---	---	1,081.90		
7	1,118.43	0.90	1,119.33	42.10	35.85 - 42.10	5-Nov-96	36.16	---	---	1,083.17	> 1E-04	Sandstone
						6-Dec-96	36.32	---	---	1,083.02		
						11-Feb-97	36.57	---	---	1,082.76		
						2-Apr-97	36.63	---	---	1,082.70		
						30-Apr-97	36.14	---	---	1,083.19		
						3-Jun-97	35.79	---	---	1,083.54		
						4-Jul-97	35.68	---	---	1,083.65		
						26-Aug-97	36.61	---	---	1,082.72		
						19-Nov-97	35.78	---	---	1,083.55		
						22-May-98	36.53	---	---	1,082.80		
						21-Aug-98	35.01	---	---	1,084.32		
						11-May-99	36.54	---	---	1,082.79		
						26-Jan-00	35.94	---	---	1,083.39		
						7-Apr-00	36.36	---	---	1,082.97		
						18-Sep-00	35.28	---	---	1,084.05		
						25-Apr-01	36.11	---	---	1,083.22		
8	1,118.96	0.87	1,119.83	38.33	32.10 - 38.33	5-Nov-96	35.64	---	---	1,084.19	> 1E-04	Sandstone
						6-Dec-96	35.82	---	---	1,084.02		
						11-Feb-97	36.11	---	---	1,083.72		
						2-Apr-97	36.18	---	---	1,083.66		
						30-Apr-97	35.73	---	---	1,084.10		
						3-Jun-97	35.42	---	---	1,084.41		
						4-Jul-97	35.18	---	---	1,084.65		
						26-Aug-97	36.01	---	---	1,083.82		
						19-Nov-97	35.17	---	---	1,084.67		
						22-May-98	36.07	---	---	1,083.76		
						21-Aug-98	34.31	---	---	1,085.52		
						11-May-99	36.07	---	---	1,083.77		
						26-Jan-00	35.37	---	---	1,084.46		
						7-Apr-00	35.85	---	---	1,083.98		
						18-Sep-00	34.63	---	---	1,085.20		
						25-Apr-01	35.69	---	---	1,084.14		

Table A-1

Monitoring Well Installation Details, Datum/Groundwater Surface Elevations And Hydraulic Conductivities

Monitoring Station	Ground Elevation (masl)	Stick-Up PVC Pipe (m)	Datum Elevation (top of PVC casing) (masl)	Depth of MW (below ground) (m)	Depth Interval of Sand (below ground) (m)	Date (d-m-y)	Depth To Water Below Datum (m)	Depth To Product Below Datum (m)	Apparent Condensate Thickness (m)	Groundwater Surface Elevation (masl)	Hydraulic Conductivity (m/s)	Lithology
9	1,108.39	0.69	1,109.08	31.93	25.70 - 31.93	5-Nov-96	24.68	---	---	1,084.40	> 1E-04	Sandstone
						6-Dec-96	24.86	---	---	1,084.22		
						11-Feb-97	25.14	---	---	1,083.94		
						2-Apr-97	25.23	---	---	1,083.85		
						30-Apr-97	24.82	---	---	1,084.26		
						3-Jun-97	24.53	---	---	1,084.55		
						4-Jul-97	24.26	---	---	1,084.82		
						26-Aug-97	25.30	---	---	1,083.78		
						19-Nov-97	24.20	---	---	1,084.88		
						22-May-98	25.11	---	---	1,083.97		
						11-May-99	25.12	---	---	1,083.97		
						26-Jan-00	24.38	---	---	1,084.70		
						18-Sep-00	23.63	---	---	1,085.45		
						25-Apr-01	24.76	---	---	1,084.32		
10	1,094.07	0.67	1,094.74	10.66	7.20 - 10.66	5-Nov-96	9.24	---	---	1,085.50	1.6E-08	Sandstone
						6-Dec-96	9.51	---	---	1,085.23		
						11-Feb-97	9.68	---	---	1,085.06		
						2-Apr-97	9.37	---	---	1,085.37		
						30-Apr-97	9.28	---	---	1,085.46		
						3-Jun-97	9.25	---	---	1,085.49		
						4-Jul-97	9.70	---	---	1,085.04		
						26-Aug-97	9.87	---	---	1,084.87		
						19-Nov-97	9.37	---	---	1,085.38		
						22-May-98	10.05	---	---	1,084.69		
						11-May-99	10.18	---	---	1,084.57		
						28-Jan-00	9.58	---	---	1,085.16		
						19-Sep-00	8.86	---	---	1,085.88		
						25-Apr-01	9.61	---	---	1,085.13		
11	1,119.02	0.67	1,119.69	42.08	35.80 - 42.08	5-Nov-96	37.23	---	---	1,082.46	> 1E-04	Sandstone
						6-Dec-96	37.44	---	---	1,082.25		
						11-Feb-97	37.69	---	---	1,082.00		
						2-Apr-97	37.56	---	---	1,082.14		
						30-Apr-97	37.28	---	---	1,082.41		
						3-Jun-97	36.95	---	---	1,082.74		
						4-Jul-97	36.84	---	---	1,082.85		
						26-Aug-97	37.00	---	---	1,082.69		
						19-Nov-97	36.90	---	---	1,082.79		
						22-May-98	37.66	---	---	1,082.04		
						11-May-99	37.65	---	---	1,082.04		
						26-Jan-00	37.04	---	---	1,082.65		
						18-Sep-00	36.35	---	---	1,083.34		
						25-Apr-01	37.27	---	---	1,082.43		

Table A-1

Monitoring Well Installation Details, Datum/Groundwater Surface Elevations And Hydraulic Conductivities

Monitoring Station	Ground Elevation (masl)	Stick-Up PVC Pipe (m)	Datum Elevation (top of PVC casing) (masl)	Depth of MW (below ground) (m)	Depth Interval of Sand (below ground) (m)	Date (d-m-y)	Depth To Water Below Datum (m)	Depth To Product Below Datum (m)	Apparent Condensate Thickness (m)	Groundwater Surface Elevation (masl)	Hydraulic Conductivity (m/s)	Lithology
12	1,117.09	0.78	1,117.87	47.02	40.80 - 47.02	5-Nov-96	40.49	---	---	1,077.38	> 1E-04	Sandstone
						6-Dec-96	40.98	---	---	1,076.89		
						11-Feb-97	41.22	---	---	1,076.65		
						2-Apr-97	41.39	---	---	1,076.48		
						30-Apr-97	41.10	---	---	1,076.77		
						3-Jun-97	40.87	---	---	1,077.00		
						4-Jul-97	40.96	---	---	1,076.91		
						26-Aug-97	40.87	---	---	1,077.00		
						19-Nov-97	41.26	---	---	1,076.61		
						21-May-98	40.28	---	---	1,077.59		
						11-May-99	40.10	---	---	1,077.77		
						27-Jan-00	39.49	---	---	1,078.38		
						18-Sep-00	38.97	---	---	1,078.90		
						25-Apr-01	39.47	---	---	1,078.40		
13	1,115.76	0.92	1,116.68	34.90	28.67 - 34.90	5-Nov-96	31.41	---	---	1,085.27	> 1E-04	Sandstone
						6-Dec-96	31.48	---	---	1,085.21		
						11-Feb-97	31.78	---	---	1,084.90		
						2-Apr-97	31.64	---	---	1,085.04		
						30-Apr-97	31.22	---	---	1,085.46		
						27-May-97	N/M	---	---	N/M		
						3-Jun-97	30.93	---	---	1,085.75		
						4-Jul-97	30.81	---	---	1,085.87		
						26-Aug-97	31.02	---	---	1,085.66		
						19-Nov-97	31.16	---	---	1,085.52		
						22-May-98	31.64	31.63	0.010	1,085.05		
						11-May-99	31.83	---	---	1,084.85		
						28-Jan-00	31.37	31.37	0.002	1,085.31		
						19-Sep-00	30.78	---	---	1,085.90		
						25-Apr-01	31.73	---	---	1,084.96		
14	1,116.22	0.67	1,116.89	33.90	30.30 - 33.90	20-May-98	31.71	---	---	1,085.18	> 1E-04	Sandstone
						11-May-99	31.76	---	---	1,085.14		
						28-Jan-00	31.30	---	---	1,085.59		
						18-Sep-00	30.62	---	---	1,086.27		
						25-Apr-01	31.29	---	---	1,085.60		

Table A-1

Monitoring Well Installation Details, Datum/Groundwater Surface Elevations And Hydraulic Conductivities

Monitoring Station	Ground Elevation (masl)	Stick-Up PVC Pipe (m)	Datum Elevation (top of PVC casing) (masl)	Depth of MW (below ground) (m)	Depth Interval of Sand (below ground) (m)	Date (d-m-y)	Depth To Water Below Datum (m)	Depth To Product Below Datum (m)	Apparent Condensate Thickness (m)	Groundwater Surface Elevation (masl)	Hydraulic Conductivity (m/s)	Lithology
15	1,118.33	0.68	1,119.01	42.40	35.00 - 42.40	20-May-98	34.57	---	---	1,084.44	1.6E-08	Sandstone
						11-May-99	34.01	---	---	1,085.00		
						28-Jan-00	34.12	---	---	1,084.89		
						18-Sep-00	33.44	---	---	1,085.57		
						25-Apr-01	34.17	---	---	1,084.84		
16	1,111.60	0.75	1,112.35	31.35	27.40 - 31.35	20-May-98	27.31	---	---	1,085.05	> 1E-04	Sandstone
						11-May-99	27.34	---	---	1,085.01		
						27-Jan-00	26.86	---	---	1,085.49		
						18-Sep-00	26.18	---	---	1,086.17		
						25-Apr-01	26.91	---	---	1,085.44		
17	1,094.07	0.69	1,094.76	19.50	16.20 - 19.50	21-May-98	16.62	---	---	1,078.14	> 1E-04	Sandstone
						11-May-99	16.66	---	---	1,078.10		
						27-Jan-00	16.40	---	---	1,078.36		
						19-Sep-00	16.04	---	---	1,078.72		
						25-Apr-01	16.22	---	---	1,078.54		
18	1,116.88	0.77	1,117.65	40.00	33.20 - 40.00	21-May-98	39.88	---	---	1,077.77	NT	Sandstone
						11-May-99	39.87	---	---	1,077.78		
						27-Jan-00	39.66	---	---	1,077.99		
						18-Sep-00	39.30	---	---	1,078.35		
						25-Apr-01	39.63	---	---	1,078.03		
19	1,118.98	0.75	1,119.73	41.55	34.50 - 41.55	21-May-98	37.54	---	---	1,082.19	> 1E-04	Sandstone
						11-May-99	37.57	---	---	1,082.17		
						27-Jan-00	36.98	---	---	1,082.75		
						7-Apr-00	37.43	---	---	1,082.30		
						19-Sep-00	36.30	---	---	1,083.43		
						25-Apr-01	37.12	---	---	1,082.61		
20	1,115.54	0.80	1,116.34	32.00	27.00 - 32.00	19-Sep-00	29.85	---	---	1,086.49	N/T	Sandstone
						25-Apr-01	30.77	---	---	1,085.57		
21	1,116.12	0.90	1,117.02	32.50	27.50 - 32.50	19-Sep-00	30.41	---	---	1,086.61	N/T	Sandstone
						25-Apr-01	32.32	---	---	1,084.70		
22	1,116.06	0.85	1,116.91	32.50	27.50 - 32.50	19-Sep-00	29.35	---	---	1,087.56	N/T	Sandstone
						25-Apr-01	30.27	---	---	1,086.64		
23	N/M	N/M	N/M	N/M	N/M	19-Sep-00	31.50	---	---	N/M	N/T	Sandstone
						25-Apr-01	32.16	---	---	N/M		

Table A-1

Monitoring Well Installation Details, Datum/Groundwater Surface Elevations And Hydraulic Conductivities

Monitoring Station	Ground Elevation (masl)	Stick-Up PVC Pipe (m)	Datum Elevation (top of PVC casing) (masl)	Depth of MW (below ground) (m)	Depth Interval of Sand (below ground) (m)	Date (d-m-y)	Depth To Water Below Datum (m)	Depth To Product Below Datum (m)	Apparent Condensate Thickness (m)	Groundwater Surface Elevation (masl)	Hydraulic Conductivity (m/s)	Lithology
24	N/M	N/M	N/M	N/M	N/M	19-Sep-00	31.69	---	---	N/M	N/T	Sandstone
						25-Apr-01	32.60	---	---	N/M		
25	1,117.96	0.69	1,118.65	43.20	34.80 - 43.20	28-Jun-00	36.23	---	---	1,082.42	N/M	Sandstone
						18-Sep-00	37.24	---	---	1,081.41		
						25-Apr-01	37.92	---	---	1,080.73		
26	N/M	N/M	N/M	N/M	N/M	25-Apr-01	30.46	---	---	N/M	N/M	
27	N/M	N/M	N/M	N/M	N/M	25-Apr-01	35.90	---	---	N/M	N/M	
28	N/M	N/M	N/M	N/M	N/M	25-Apr-01	N/M	---	---	N/M	N/M	
						26-Apr-01	31.82	30.73	1.085	N/M		
28 (Post Purge)						26-Apr-01	30.90	30.88	0.017			
MW9902	1,117.47	0.89	1,118.36	36.58	26.82 - 36.58	28-Jan-00	33.24	---	---	1,085.12	NT	Sandstone
						28-Jun-00	33.51	---	---	1,084.85		
						18-Sep-00	32.51	---	---	1,085.85		
						25-Apr-01	33.30	---	---	1,085.06		
MW9903	1,117.04	0.91	1,117.95	38.86	28.65 - 38.86	28-Jan-00	32.59	---	---	1,085.36	NT	Sandstone
						18-Sep-00	31.88	---	---	1,086.07		
						25-Apr-01	33.65	---	---	1,084.30		
MW9905	1,118.11	1.26	1,119.37	41.15	22.86 - 41.15	28-Jan-00	33.85	---	---	1,085.52	NT	Sandstone
						18-Sep-00	33.14	---	---	1,086.23		
						25-Apr-01	33.91	---	---	1,085.46		
EW-1	1,114.74	0.90	1,115.64	37.80	31.00 - 37.80		N/M	---	---	N/M	2.3E-05	

NOTES:

1. Data may be entered to the nearest mm, but are reported above to the nearest cm.
Apparent rounding errors may occasionally occur in calculated fields (e.g., Groundwater Surface Elevation).

2. Where free product is present, Groundwater Surface Elevation is calculated as
*Groundwater Surface = Datum Elevation - Depth to Water + Product Specific Density * Product Thickness.*

3. N/M - Denotes not measured.

Table A-2
Water Quality: Field Measured Parameters

Monitoring Station	Date (d-m-y)	Temp (°C)	Electrical Conductivity (µS/cm)	pH (unit)	Eh (mV)	DO (mg/L)	Comments
1	31-Jul-96	6.2	585	7.23	---	---	
	29-Aug-96	---	---	---	---	---	Water levels only, no sample collected
	5-Nov-96	5.5	560	7.58	---	---	
	6-Dec-96	---	---	---	---	---	Water levels only, no sample collected
	19-Nov-97	4.5	490	7.36	---	---	
	22-May-98	5.0	510	7.50	---	3.4	
	26-Jan-00	4.5	507	6.89	7	4.2	
	18-Sep-00	7.0	540	7.55	-32	0.4	
	25-Apr-01	8.3	502	7.60	31	3.6	No hydrocarbon odour/sheen
2	31-Jul-96	6.7	594	7.69	---	---	
	29-Aug-96	---	---	---	---	---	Hydrocarbon sheen, odour
	5-Nov-96	5.5	570	7.60	---	---	Hydrocarbon droplets
	6-Dec-96	---	---	---	---	---	Water levels only, no sample collected
	19-Nov-97	5.0	550	7.25	---	---	
	22-May-98	5.8	580	7.35	---	---	Odour
	21-Aug-98	7.6	518	7.07	---	0.5	Faint hydrocarbon odour
	26-Jan-00	4.7	564	7.10	12	0.5	Moderate hydrocarbon odour
	19-Sep-00	7.0	561	7.35	---	---	Hydrocarbon odour
	25-Apr-01	13.1	571	7.51	---	---	Hydrocarbon odour
3	31-Jul-96	6.7	742	7.36	---	---	Diesel odour, sheen on water
	29-Aug-96	---	---	---	---	---	Hydrocarbon sheen, odour
	5-Nov-96	5.5	588	7.85	---	---	Hydrocarbon odour
	6-Dec-96	---	---	---	---	---	Water levels only, no sample collected
	19-Nov-97	---	---	---	0	---	
	22-May-98	---	---	---	---	---	Water levels only, no sample collected
	28-Jan-00	---	---	---	---	---	Not sampled
	19-Sep-00	8.2	730	7.00	---	---	Hydrocarbon sheen, odour - Free-Product 0.39 m
	23-Oct-00	6.8	607	6.8	-52	<1	Free product 0.26 m
	25-Apr-01	---	---	---	---	---	Not sampled, removed 0.4L with skimmer bailer
	22-May-01	10.8	593	7.97	-17.8	---	Removed 0.4 L with skimmer bailer, additional 0.145 m of free-product remaining
4	31-Jul-96	6.6	702	7.40	---	---	
	29-Aug-96	---	---	---	---	---	Hydrocarbon sheen, odour
	5-Nov-96	5.4	589	7.56	---	---	Hydrocarbon odour
	6-Dec-96	---	---	---	---	---	Water levels only, no sample collected
	19-Nov-97	---	---	---	0	---	
	22-May-98	---	---	---	---	---	Not sampled, free product
	28-Jan-00	---	---	---	---	---	Not sampled, free product
	19-Sep-00	---	---	---	---	---	Not sampled, free product
	25-Apr-01	---	---	---	---	---	Not sampled, removed 0.3L with skimmer bailer
	22-May-01	7.8	627	6.93	-48.8	---	Removed 0.25 L with skimmer bailer, no free-product remaining
5	5-Jul-96	8.0	497	7.23	---	---	Diesel odour
	19-Jul-96	---	---	---	5	---	
	5-Nov-96	---	---	---	---	---	Not sampled
	6-Dec-96	---	---	---	---	---	Could not access piezometer
	19-Nov-97	4.2	616	7.32	---	---	
	22-May-98	6.0	538	7.40	---	---	Odour, sheen
	27-Jan-00	4.2	760	7.33	---	---	Hydrocarbon sheen, strong odour
	18-Sep-00	8.7	628	7.32	---	---	Hydrocarbon sheen, odour
	25-Apr-01	5.3	527	7.42	138	0.4	Hydrocarbon sheen, odour

Table A-2
Water Quality: Field Measured Parameters

Monitoring Station	Date (d-m-y)	Temp (°C)	Electrical Conductivity (µS/cm)	pH (unit)	Eh (mV)	DO mg/L)	Comments
6	5-Nov-96	5.4	566	7.36	---	---	Clean
	6-Dec-96	---	---	---	---	---	Water levels only, no sample collected
	19-Nov-97	4.6	551	7.30	---	---	
	21-May-98	4.3	526	7.43	130	0.7	
	26-Jan-00	4.6	536	6.49	54	0.5	
	18-Sep-00	6.0	567	7.22	6	0.2	
	25-Apr-01	3.4	515	7.44	47	0.4	
7	5-Nov-96	5.5	534	7.79	---	---	Hydrocarbon droplets
	6-Dec-96	---	---	---	---	---	Water levels only, no sample collected
	19-Nov-97	4.5	550	7.25	---	---	
	22-May-98	4.7	555	7.45	-50	0.7	
	21-Aug-98	7.9	358	7.18	---	0.4	
	26-Jan-00	4.5	537	7.37	73	0.4	
	7-Apr-00	6.3	527	7.00	---	---	Cloudy, slight diesel odour
	18-Sep-00	5.2	608	7.52	-58	0.6	Hydrocarbon odour
	25-Apr-01	3.5	532	7.41	12	0.3	No hydrocarbon odour/sheen
	22-May-01	9	551	7.03	-20	6	No hydrocarbon odour/sheen
8	5-Nov-96	5.6	560	7.80	---	---	Hydrocarbon droplets
	6-Dec-96	---	---	---	---	---	Water levels only, no sample collected
	19-Nov-97	4.7	540	7.30	---	---	
	22-May-98	4.7	570	7.40	---	1.2	
	21-Aug-98	7.7	309	7.33	---	3.8	Faint hydrocarbon odour
	26-Jan-00	4.4	527	7.01	-3	0.3	
	7-Apr-00	6.5	540	6.91	---	---	Cloudy, slight diesel odour
	18-Sep-00	5.6	568	7.39	-64	0.4	
	23-Oct-00	5.2	527	7.15	-11	1.0	
	25-Apr-01	4.8	572	7.35	49	0.3	No hydrocarbon odour/sheen
	22-May-01	9.2	---	6.9	-12.7	6	Cloudy, slight diesel odour
9	5-Nov-96	5.6	562	7.68	---	---	Clean
	6-Dec-96	---	---	---	---	---	Water levels only, no sample collected
	19-Nov-97	4.7	580	7.20	---	---	
	22-May-98	5.0	565	7.52	170	5.5	
	26-Jan-00	4.4	533	7.40	60	6.0	Waterra needs replacing
	18-Sep-00	5.0	621	7.54	1	6.3	
	25-Apr-01	3.6	545	7.48	185	6.5	No hydrocarbon odour/sheen
10	5-Nov-96	6.0	695	7.78	---	---	Clean
	6-Dec-96	---	---	---	---	---	Water levels only, no sample collected
	19-Nov-97	3.5	742	7.11	---	---	
	22-May-98	5.5	726	7.20	150	6.3	Insufficient H20 for TEH sample
	28-Jan-00	5.2	792	7.42	-14	5.0	
	19-Sep-00	6.9	806	7.23	-30	3.1	Orange water
	25-Apr-01	4.4	724	7.25	66	4.2	No hydrocarbon odour/sheen
	22-May-01	8.1					Orange deposit when measuring water level
11	5-Nov-96	5.6	546	7.65	---	---	Clean
	6-Dec-96	---	---	---	---	---	Water levels only, no sample collected
	19-Nov-97	4.5	568	7.31	---	---	
	22-May-98	6.5	558	7.54	200	4.5	
	26-Jan-00	4.0	523	7.26	360	5.9	
	18-Sep-00	5.1	594	7.39	9	5.4	
	25-Apr-01	3.7	544	7.46	246	5.3	No hydrocarbon odour/sheen

Table A-2
Water Quality: Field Measured Parameters

Monitoring Station	Date (d-m-y)	Temp (°C)	Electrical Conductivity (µS/cm)	pH (unit)	Eh (mV)	DO (mg/L)	Comments
12	5-Nov-96	5.6	554	7.75	---	---	Clean
	6-Dec-96	---	---	---	---	---	Water levels only, no sample collected
	19-Nov-97	4.5	586	7.25	---	---	Need to fish waterra out of well
	21-May-98	4.4	610	7.45	100	1.7	
	27-Jan-00	4.8	590	7.19	92	4.0	
	18-Sep-00	4.8	607	7.56	19	2.7	
	25-Apr-01	4.2	572	7.49	24	3.1	No hydrocarbon odour/sheen, replaced foot valve
13	5-Nov-96	5.5	555	7.68	---	---	Hydrocarbon droplets
	6-Dec-96	---	---	---	---	---	Water levels only, no sample collected
	19-Nov-97	5.0	905	7.20	---	---	Needs foot valve
	22-May-98	---	---	---	---	---	Not sampled
	28-Jan-00	---	---	---	---	---	0.002m of product, not sampled
	19-Sep-00	7.3	1,053	7.19	---	---	Hydrocarbon sheen, odour
	25-Apr-01	---	---	---	---	---	Hydrocarbon odour present, not sampled
14	20-May-98	5.0	530	7.51	-635	0.7	
	28-Jan-00	4.7	537	7.39	-5	4.6	
	18-Sep-00	4.9	536	7.59	-33	6.2	
	25-Apr-01	8.7	527	7.65	181	4.0	No hydrocarbon odour/sheen
15	20-May-98	4.5	470	7.85	-260	3.0	Silty - not cleaning up
	28-Jan-00	4.2	555	7.16	---	0.4	Eh probe malfunction, no lock
	18-Sep-00	5.2	550	7.49	-6	2.4	Silty sample
	25-Apr-01	5.0	538	7.82	40	4.5	No hydrocarbon odour/sheen
16	20-May-98	5.3	505	7.36	-100	4.2	
	27-Jan-00	4.6	---	---	-5	4.2	Waterra broken at 5mbgs
	18-Sep-00	5.4	534	7.41	-22	0.5	
	23-Oct-00	6.2	501	7.30	-19	5.6	
	25-Apr-01	7.6	523	7.49	149	10.2	No hydrocarbon odour/sheen
	22-May-01	9.6	550	7.22	-19.6	3	
17	21-May-98	4.7	540	7.43	160	4.0	
	27-Jan-00	4.7	540	7.36	6	0.9	
	19-Sep-00	5.6	554	7.62	-30	2.7	
	25-Apr-01	5.0	532	7.49	166	8.5	
18	21-May-98	4.6	---	---	-185	3.5	Not sampled
	27-Jan-00	---	---	---	---	---	Insufficient water to sample
	18-Sep-00	5.9	578	7.46	6	3.7	
	25-Apr-01	5.8	575	7.38	99	8.7	Silty, no hydrocarbon odour/sheen, replaced Waterra

Table A-2
Water Quality: Field Measured Parameters

Monitoring Station	Date *(d-m-y)*	Temp *(°C)*	Electrical Conductivity *(µS/cm)*	pH *(unit)*	Eh *(mV)*	DO *(mg/L)*	Comments
19	21-May-98	6.0	610	7.35	-60	3.7	
	27-Jan-00	6.1	599	7.38	11	0.5	
	7-Apr-00	6.5	550	6.57	---	---	Cloudy, slight diesel odour
	19-Sep-00	6.3	634	7.38	-34	0.9	
	25-Apr-01	5.3	574	7.23	22	0.3	No hydrocarbon odour/sheen
	22-May-01	8.1	578	7.3	4.1	4.5	
20	19-Sep-00	9.9	790	7.13	---	---	
	25-Apr-01	13.5	899	7.17	---	---	Hydrocarbon odour/sheen
21	19-Sep-00	7.2	667	7.38	---	---	
	25-Apr-01	8.3	711	7.11	---	---	Slight hydrocarbon odour, needs Waterra
22	19-Sep-00	9.8	708	7.01	---	---	
	25-Apr-01	8.5	688	7.19	---	---	Slight hydrocarbon odour, needs Waterra
23	19-Sep-00	6.5	603	7.28	---	---	Hydrocarbon odour
	25-Apr-01	6.5	861	6.89	63	0.4	No hydrocarbon odour/sheen
24	19-Sep-00	6.8	559	7.50	---	---	
	25-Apr-01	8.1	535	7.41	142	2.4	No hydrocarbon odour/sheen
25	28-Jun-00	8.5	435	7.57	---	---	
	18-Sep-00	5.5	616	7.48	-16	---	
	25-Apr-01	3.3	561	7.42	46	1.1	
26	25-Apr-01	4.9	545	7.46	252	4.8	
27	25-Apr-01	3.8	551	7.45	251	4.8	White fibrous/sinuous debris at tip of probes - PVC shavings?, no HC odour/sheen
28	25-Apr-01	14.6	619	7.11	---	---	Hydrocarbon odour/sheen, hot day
	26-Apr-01	---	---	---	---	---	Removed 1.5L free product, 1.5L emulstion, 1L water
MW9902	28-Jan-00	3.6	541	7.04	114	0.9	Eh value 114mV suspect
	28-Jun-00	6.7	483	7.30	---	---	HC sheen present, no measurable product
	18-Sep-00	5.4	568	7.39	-41	2.5	
	25-Apr-01	7.6	525	7.44	71	3.6	Hydrocarbon odour/sheen present
MW9903	27-Jan-00	4.2	546	7.37	1	3.5	
	18-Sep-00	6.1	552	7.50	-32	5.2	
	25-Apr-01	7.1	540	7.38	134	9.6	No hydrocarbon odour/sheen
MW9905	28-Jan-00	4.2	547	7.34	8	5.1	
	18-Sep-00	5.9	546	7.52	38	6.5	
	25-Apr-01	10.9	550	7.38	169	2.7	No hydrocarbon odour/sheen

NOTES: *1. Electrical conductivity values standardized to 25°C.*

Table A-3
Water Quality: Indicator Parameter Concentrations

Monitoring Station	Date (d-m-y)	Chloride:D (mg/L)	Sulphate:D (mg/L)	TDS-calculated (mg/L)	Fluoride (mg/L)	NO_2+NO_3 as N (mg/L)	Iron:D (mg/L)	Manganese:D (mg/L)	Sodium:D (mg/L)
Canadian Drinking Water Guidelines		*250*	*500*	*500*	*1.5*	*10*	*0.3*	*0.05*	*200*
1	31-Jul-96	0.6	7.8	310	0.15	0.290	<0.01	0.026	9.36
	6-Nov-96	1.2	7.9	315	0.14	0.302	<0.01	0.007	10.1
	19-Nov-97	1.3	7.3	301	0.15	0.325	0.05	0.002	8.03
	21-May-98	1.6	7.4	298	—	0.277	<0.01	<0.001	8.6
	26-Jan-00	1.9	7.6	309	—	0.36	<0.01	<0.01	9
	18-Sep-00	1.9	7.3	307	—	0.510	<0.01	<0.004	8.5
	25-Apr-01	2.6	7.8	300	—	0.354	<0.01	0.028	8.9
2	31-Jul-96	8.0	7.4	342	0.15	0.127	<0.01	0.065 [1]	10.8
	6-Nov-96	5.1	6.1	328	0.13	0.084	<0.01	0.024	9.90
	19-Nov-97	6.4	5.6	329	0.13	0.066	<0.01	0.039	8.24
	22-May-98	10.9	6.5	329	—	0.091	<0.01	0.012	8.8
	26-Jan-00	7.9	5.9	341	—	0.14	<0.01	0.03	9
	19-Sep-00	12.7	6.6	344	—	0.282	<0.01	0.023	9.1
	25-Apr-01	6.9	6.8	319	—	0.026	<0.01	0.041	8.8
3	31-Jul-96	8.2	0.7	449	0.13	<0.003	<0.01	0.537 [1]	8.91
	6-Nov-96	3.0	1.0	372	0.11	0.004	<0.01	0.229 [1]	9.35
	19-Sep-00	7.9	0.4	439	—	<0.003	0.04	0.416 [1]	7.6
(duplicate)	19-Sep-00	7.5	0.3	429	—	<0.003	0.03	0.413 [1]	7.7
4	31-Jul-96	6.9	0.9	409	0.12	<0.003	<0.01	0.373 [1]	8.71
	6-Nov-96	2.8	0.9	364	0.12	0.004	<0.01	0.151 [1]	8.85
5	5-Jul-96	1.4	6.9	316	0.11	0.047	<0.01	0.171 [1]	8.21
	19-Jul-96	3.9	7.6	318	0.15	0.209	<0.01	0.146 [1]	9.04
	19-Nov-97	5.1	7.1	353	0.13	0.010	<0.01	0.275 [1]	8.46
	22-May-98	2.6	8.9	317	—	0.005	<0.01	0.238 [1]	8.6
	27-Jan-00	3.9	76.7	476	—	<0.05	<0.01	0.42 [1]	67
	18-Sep-00	5.8	12.2	356	—	<0.003	<0.01	0.237 [1]	13.0
	25-Apr-01	2.9	12.2	312	—	0.014	<0.01	0.250 [1]	11.8
6	5-Nov-96	2.0	8.7	325	0.20	0.064	<0.01	0.040	10.5
	19-Nov-97	3.1	9.5	325	0.12	0.033	<0.01	0.003	8.54
	22-May-98	2.2	8.4	310	—	0.152	<0.01	0.005	8.2
	26-Jan-00	2.1	10.7	331	—	0.06	<0.01	0.01	8
	18-Sep-00	3.4	9.1	328	—	0.131	<0.01	<0.004	8.6
	25-Apr-01	2.9	9.4	306	—	0.096	<0.01	<0.004	8.5
7	6-Nov-96	2.0	12.1	320	0.14	0.004	<0.01	0.032	8.63
	19-Nov-97	3.0	9.9	334	0.13	0.009	<0.01	0.104 [1]	7.5
	22-May-98	2.8	11.6	314	—	0.018	<0.01	0.036	7.4
	26-Jan-00	2.2	11.4	345	—	<0.05	<0.01	0.02	8
	7-Apr-00	1.2	12.9	325	(0.09)	<0.003	<0.01	0.103 [1]	7.8
(duplicate)	7-Apr-00	2.2	13.9	315	<0.1	0.22	<0.01	0.11 [1]	7
	18-Sep-00	3.8	10.0	335	—	<0.003	<0.01	0.016	7.5
	25-Apr-01	3.0	13.2	314	—	<0.003	<0.01	0.071 [1]	7.6
8	6-Nov-96	1.5	18.8	340	0.16	0.130	<0.01	0.020	8.03
	19-Nov-97	1.6	15.7	331	0.14	0.130	<0.01	0.052 [1]	6.91
	22-May-98	2.9	16.2	338	—	0.039	<0.01	0.022	6.9
	26-Jan-00	1.8	39.0	352	—	0.25	<0.01	0.07 [1]	7
	7-Apr-00	1.0	20.7	340	0.12	0.146	<0.01	0.003	7.1
(duplicate)	7-Apr-00	2.0	19.8	334	0.1	0.61	<0.01	0.02	6
	19-Sep-00	2.5	15.0	326	—	0.169	0.03	<0.004	6.9
(duplicate)	19-Sep-00	2.9	13.9	325	—	0.172	0.02	<0.004	6.8
	25-Apr-01	3.3	11.8	333	—	0.006	<0.01	0.024	7.1

Table A-3
Water Quality: Indicator Parameter Concentrations

Monitoring Station	Date *(d-m-y)*	Chloride:D (mg/L)	Sulphate:D (mg/L)	TDS-calculated (mg/L)	Fluoride (mg/L)	NO_2+NO_3 as N (mg/L)	Iron:D (mg/L)	Manganese:D (mg/L)	Sodium:D (mg/L)
Canadian Drinking Water Guidelines		*250*	*500*	*500*	*1.5*	*10*	*0.3*	*0.05*	*200*
9	5-Nov-96	1.8	51.3	364	0.19	0.201	<0.01	0.019	7.64
	19-Nov-97	1.5	45.0	366	0.18	0.184	<0.01	0.001	6.63
	22-May-98	2.3	53.1	347	---	0.190	<0.01	0.001	6.5
	26-Jan-00	2.0	14.1	305	---	0.08	<0.01	<0.01	6
	18-Sep-00	1.9	38.8	354	---	0.211	<0.01	<0.004	6.3
	25-Apr-01	2.5	47.5	334	---	0.323	<0.01	<0.004	6.3
10	5-Nov-96	1.1	8.7	458	0.19	0.019	<0.01	0.228[1]	5.80
	19-Nov-97	<0.5	7.3	454	0.16	0.085	<0.01	0.002	3.97
	22-May-98	0.6	7.3	442	---	0.084	<0.01	<0.001	4.1
	28-Jan-00	0.5	5.7	469	---	0.08	<0.01	<0.01	4
	19-Sep-00	<0.5	3.7	485	---	0.021	<0.01	0.028	4.1
	25-Apr-01	<0.5	5.6	444	---	0.104	<0.01	<0.004	4.0
11	5-Nov-96	1.9	48.6	366	0.20	0.254	<0.01	0.010	7.88
	19-Nov-97	1.8	45.0	358	0.18	0.270	<0.01	<0.001	6.55
	22-May-98	2.1	47.2	348	---	0.213	<0.01	<0.001	6.8
	26-Jan-00	0.5	84.0	381	---	<0.05	<0.01	<0.01	7
	18-Sep-00	2.8	39.4	341	---	0.408	<0.01	<0.004	6.8
	25-Apr-01	2.4	48.1	335	---	0.289	<0.01	<0.004	6.7
12	5-Nov-96	1.4	44.7	361	0.19	0.168	<0.01	0.028	8.74
	19-Nov-97	1.6	42.8	363	0.18	0.176	<0.01	0.014	11.8
	20-May-98	1.9	41.0	355	---	0.154	<0.01	0.009	9.4
	27-Jan-00	1.6	38.8	329	---	0.22	<0.01	<0.01	7
	18-Sep-00	2.1	39.1	350	---	0.178	<0.01	<0.004	7.1
	25-Apr-01	2.0	41.0	343	---	0.238	<0.01	<0.004	7.1
13	6-Nov-96	49.7	12.5	491	0.19	0.074	<0.01	0.039	15.4
	19-Nov-97	39.0	13.2	517[1]	0.14	0.009	<0.01	0.383[1]	10.5
	19-Sep-00	69.3	3.9	599[1]	---	0.030	<0.01	0.336[1]	11.9
14	20-May-98	1.9	7.4	306	---	0.261	<0.01	0.058[1]	9.6
	28-Jan-00	2.2	7.3	311	---	0.32	<0.01	<0.01	9
	19-Sep-00	2.5	6.7	306	---	0.476	<0.01	<0.004	8.4
	25-Apr-01	2.9	7.7	300	---	0.343	<0.01	<0.004	9.1
15	20-May-98	3.4	24.8	270	---	0.357	<0.01	0.072[1]	14.7
	28-Jan-00	1.8	32.5	332	---	0.06	<0.01	0.07[1]	19
	18-Sep-00	2.2	16.9	310	---	0.026	<0.01	0.036	13.0
	25-Apr-01	2.2	17.9	300	---	0.043	<0.01	0.050	11.6
16	20-May-98	2.1	6.3	308	---	0.246	<0.01	0.014	8.6
	18-Sep-00	2.1	6.3	305	---	0.371	<0.01	<0.004	8.7
	25-Apr-01	3.4	7.9	297	---	0.271	<0.01	0.068[1]	8.6
17	20-May-98	1.9	8.5	310	---	0.393	<0.01	0.005	7.3
	27-Jan-00	2.0	11.4	305	---	0.07	<0.01	<0.01	7
	19-Sep-00	2.1	7.8	316	---	0.369	<0.01	<0.004	7.2
	25-Apr-01	2.6	10.7	305	---	0.204	<0.01	0.040	7.9
18	18-Sep-00	1.9	30.7	330	---	0.359	<0.01	0.021	6.6
	25-Apr-01	2.2	34.0	342	---	0.205	<0.01	0.011	6.8
19	20-May-98	4.7	11.6	346	---	0.006	<0.01	0.171[1]	7.0
	27-Jan-00	2.1	13.0	348	---	<0.05	<0.01	0.16[1]	7
	7-Apr-00	1.2	12.3	344	(0.09)	0.014	<0.01	0.149[1]	7.0
(duplicate)	7-Apr-00	2.4	12.6	338	<0.1	0.17	<0.01	0.14[1]	6
	19-Sep-00	4.7	8.1	360	---	0.008	<0.01	0.148[1]	7.4
	25-Apr-01	3.4	12.4	336	---	<0.003	<0.01	0.159[1]	6.9

Table A-3
Water Quality: Indicator Parameter Concentrations

Monitoring Station	Date (d-m-y)	Chloride:D (mg/L)	Sulphate:D (mg/L)	TDS-calculated (mg/L)	Fluoride (mg/L)	NO_2+NO_3 as N (mg/L)	Iron:D (mg/L)	Manganese:D (mg/L)	Sodium:D (mg/L)
Canadian Drinking Water Guidelines		*250*	*500*	*500*	*1.5*	*10*	*0.3*	*0.05*	*200*
20	19-Sep-00	9.2	7.5	457	---	(0.003)	<0.01	3.24 ^{1}	11.7
	25-Apr-01	7.7	1.9	479	---	<0.003	0.22	1.18 ^{1}	11.1
(duplicate)	25-Apr-01	7.3	1.3	475	---	<0.003	2.46 ^{1}	1.10 ^{1}	10.9
21	19-Sep-00	6.2	12.0	383	---	<0.003	<0.01	1.37 ^{1}	11.5
	25-Apr-01	6.0	0.9	392	---	<0.003	0.02	1.28 ^{1}	9.2
22	19-Sep-00	5.9	63.0	457	---	0.006	0.02	0.980 ^{1}	86.0
	25-Apr-01	5.0	3.6	382	---	<0.003	1.43 ^{1}	0.806 ^{1}	29.0
23	19-Sep-00	5.5	20.3	357	---	(0.003)	<0.01	0.086 ^{1}	8.3
	25-Apr-01	8.5	13.5	502 ^{1}	---	<0.003	0.09	0.306 ^{1}	7.9
24	19-Sep-00	2.5	6.9	319	---	0.498	<0.01	<0.004	10.0
	25-Apr-01	2.8	7.8	301	---	0.509	<0.01	<0.004	8.5
25	28-Jun-00	2.8	42.7	311	0.23	<0.003	<0.01	0.206 ^{1}	8.1
	18-Sep-00	2.2	30.4	352	---	0.021	<0.01	0.762 ^{1}	8.1
	25-Apr-01	2.1	33.2	340	---	0.159	<0.01	0.027	6.8
26	7-Mar-01	2.4	28.6	331	---	0.301	<0.01	0.009	6.8
	25-Apr-01	2.6	26.0	323	---	0.387	<0.01	(0.005)	6.6
27	7-Mar-01	3.2	36.5	334	---	0.303	<0.01	0.022	6.3
	25-Apr-01	2.2	37.6	333	---	0.321	<0.01	(0.005)	6.7
28	8-Mar-01	24.8	6.8	342	---	0.022	<0.01	0.174 ^{1}	9.1
	25-Apr-01	23.3	7.1	332	---	<0.003	<0.01	0.140 ^{1}	9.2
MW9902	28-Jan-00	2.1	6.8	300	---	0.40	<0.01	0.02	8
	28-Jun-00	2.1	7.8	313	0.10	0.202	<0.01	0.014	8.6
	18-Sep-00	2.0	6.6	322	---	0.390	0.06	(0.004)	9.9
	25-Apr-01	2.5	7.6	294	---	0.238	<0.01	0.065 ^{1}	5.5
MW9903	27-Jan-00	2.2	6.6	300	---	0.28	<0.01	<0.01	9
	18-Sep-00	2.1	6.7	314	---	0.445	<0.01	<0.004	10.1
	25-Apr-01	3.0	7.3	290	---	0.319	<0.01	<0.004	7.9
MW9905	28-Jan-00	2.1	7.1	315	---	0.44	<0.01	<0.01	9
	18-Sep-00	1.9	7.4	317	---	0.521	<0.01	<0.004	12.1
	25-Apr-01	2.2	7.9	307	---	0.350	<0.01	0.010	9.1
EW-1	8-Sep-00	5.2	0.7	384	---	(0.005)	<0.01	0.267 ^{1}	11.1
	11-Sep-00	5.6	0.8	371	---	<0.003	<0.01	0.242 ^{1}	8.6
	14-Sep-00	4.8	1.0	365	---	<0.003	0.02	0.240 ^{1}	8.2
	21-Sep-00	3.6	4.8	360	---	<0.003	<0.01	0.257 ^{1}	9.2
	30-Sep-00	3.9	5.3	342	---	0.021	<0.01	0.198 ^{1}	7.9
Trip Blank	5-Jul-96	<0.5	0.7	2	<0.05	0.004	<0.01	0.002	0.19
	4-Feb-98	11.9	26.3	449	0.30	2.76	<0.01	<0.001	58.9

NOTES:

1. — in detail row(s) denotes parameter not analyzed

2. (0.001) Single bracketed results are values below the reliable detection level, and are subject to reduced levels of confidence.

3. Superscript ^{1} denotes values exceeding Guidelines for Canadian Drinking Water Quality (Health Canada, 1996).

4. NO_2+NO_3 as N - Limit is for Nitrate as N.

Table A-4
Water Quality: Dissolved Hydrocarbon Analyses

Monitoring Station	Date (d-m-y)	Benzene (mg/L)	Toluene (mg/L)	Ethylbenzene (mg/L)	Xylene-m&p (mg/L)	Xylene-o (mg/L)	Xylene-total (mg/L)	TPH (C_5-C_{30}) (mg/L)	TVH (C_3-C_{10}) (mg/L)	TPH (C_{11}-C_{30}) (mg/L)	TEH (C_{11}-C_{30}) (mg/L)	TEH (C_{11}-C_{31}) (mg/L)
Canadian Drinking Water Guidelines		*0.005*	*0.024*	*0.0024*	---	---	*0.3*	---	---	---	---	---
1	31-Jul-96	<0.0007	<0.0012	<0.0007	<0.0007	<0.0007	<0.0014	<0.02	---	<0.02	<0.2	---
	6-Nov-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
(duplicate)	6-Nov-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	19-Nov-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	21-May-98	<0.0009	<0.0009	<0.0009	<0.0009	<0.002	<0.0029	<0.1	---	---	<0.02	---
	26-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
2	31-Jul-96	<0.0007	<0.0012	<0.0007	0.0040	0.0020	0.006	(0.03)	---	0.79	(15.2 - 15.2)	---
	6-Nov-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	0.05	(3.06 - 3.14)	---
	19-Nov-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	0.05	0.42	---
	22-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
	21-Aug-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	0.57	---
	26-Jan-00	<0.0005	<0.0005	0.0024	---	---	0.0034	---	<0.1	---	1.1	---
	19-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	(1.1)	---
3	31-Jul-96	0.11[1]	0.16[1]	0.030[1]	0.85	0.19	1.04[1]	1.80	---	1.40	(7.71 - 7.79)	---
	6-Nov-96	0.023[1]	<0.0092	<0.0092	0.180	0.0500	0.23	0.26	---	0.12	(4.48 - 4.56)	---
	19-Sep-00	0.020[1]	0.088[1]	(0.014)[1]	0.65	0.187	0.817[1]	0.9	---	---	41.9	---
(duplicate)	19-Sep-00	0.019[1]	0.087[1]	(0.015)[1]	0.61	0.179	0.789[1]	0.9	---	---	26.5	---
	25-Apr-01	0.012[1]	0.029[1]	0.031[1]	0.973	0.248	1.22[1]	3.3	---	---	---	7.260
4	31-Jul-96	0.034[1]	<0.0036	0.011[1]	0.150	0.027	0.177	0.40	---	0.99	(3.24 - 3.34)	---
	6-Nov-96	0.017[1]	<0.0037	<0.0037	0.0700	<0.0037	(0.0700 - 0.0737)	0.15	---	0.28	(4.29 - 4.37)	---
5	5-Jul-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	19-Jul-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	1.10	---	2.40	75.5	76.0
	19-Nov-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	0.20	42	---
	22-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	11.8	---
	27-Jan-00	0.0009	<0.0005	<0.0005	---	---	0.0006	---	0.18	---	7.3	---
	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	12.9	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	38.5	---
6	5-Nov-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	19-Nov-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	22-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
	26-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---

Table A-4
Water Quality: Dissolved Hydrocarbon Analyses

Monitoring Station	Date (d-m-y)	Benzene (mg/L)	Toluene (mg/L)	Ethylbenzene (mg/L)	Xylene-m&p (mg/L)	Xylene-o (mg/L)	Xylenes-total (mg/L)	TPH (C_5-C_{10}) (mg/L)	TVH (C_5-C_{10}) (mg/L)	TPH (C_{11}-C_{12}) (mg/L)	TEH (C_{11}-C_{30}) (mg/L)	TEH (C_{11}-C_{40}) (mg/L)
Canadian Drinking Water Guidelines		*0.005*	*0.024*	*0.0024*	---	---	*0.3*	---	---	---	---	---
7	6-Nov-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	(0.23 - 0.38)	---
	19-Nov-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	0.07	0.16	---
	22-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
	21-Aug-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
	26-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	0.59	---
(duplicate)	26-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	7-Apr-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
(duplicate)	7-Apr-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	0.22	---
	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
8	6-Nov-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	(0.48 - 0.61)	---
	19-Nov-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
(duplicate)	19-Nov-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	22-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
(duplicate)	22-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	---	---
	21-Aug-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
	26-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	0.55	---
	7-Apr-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
(duplicate)	7-Apr-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	0.54	---
	19-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
(duplicate)	19-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
9	5-Nov-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	19-Nov-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	22-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
	26-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
10	5-Nov-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	19-Nov-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	22-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	---	---
	28-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	19-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
11	5-Nov-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	19-Nov-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	22-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
	26-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---

Table A-4
Water Quality: Dissolved Hydrocarbon Analyses

Monitoring Station	Date (d-m-y)	Benzene (mg/L)	Toluene (mg/L)	Ethylbenzene (mg/L)	Xylene-m&p (mg/L)	Xylene-o (mg/L)	Xylenes-total (mg/L)	TPH (C_5-C_{10}) (mg/L)	TVH (C_5-C_{10}) (mg/L)	TPH (C_{11}-C_{13}) (mg/L)	TEH (C_{11}-C_{30}) (mg/L)	TEH (C_{11}-C_{60}) (mg/L)
Canadian Drinking Water Guidelines		*0.005*	*0.024*	*0.0024*	---	---	*0.3*	---	---	---	---	---
12	5-Nov-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	19-Nov-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	20-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
	27-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
13	6-Nov-96	<0.0012	0.0027	0.0064 [1]	0.0160	0.0280	0.044	0.14	---	0.27	(1.76 - 1.87)	---
	19-Nov-97	(0.0004)	<0.0004	0.0039 [1]	0.0049	0.0044	0.0093	0.10	---	0.82	2.7	---
	19-Sep-00	<0.0004	<0.0004	0.0023	0.0034	0.0017	0.0051	<0.1	---	---	9.9	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	(0.0015)	0.0009	0.0024	<0.1	---	---	23.4	---
(duplicate)	25-Apr-01	<0.0004	<0.0004	(0.0004)	(0.0015)	0.0010	0.0025	(0.1)	---	---	41.5	---
14	20-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
	28-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	19-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.7	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
15	20-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
	28-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
16	20-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
17	20-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
	27-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	19-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.7	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
18	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
19	20-May-98	<0.0009	<0.0009	<0.0009	<0.002	<0.0009	<0.0029	<0.1	---	---	<0.02	---
	27-Jan-00	0.0007	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	0.50	---
	7-Apr-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
(duplicate)	7-Apr-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	0.41	---
	19-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---

Table A-4

Water Quality: Dissolved Hydrocarbon Analyses

Monitoring Station	Date (d-m-y)	Benzene (mg/L)	Toluene (mg/L)	Ethylbenzene (mg/L)	Xylene-m&p (mg/L)	Xylene-o (mg/L)	Xylenes-total (mg/L)	TPH (C_5-C_{10}) (mg/L)	TVH (C_5-C_{10}) (mg/L)	TPH (C_{10}-C_{12}) (mg/L)	TEH (C_{11}-C_{30}) (mg/L)	TEH (C_{11}-C_{40}) (mg/L)
Canadian Drinking Water Guidelines		*0.005*	*0.024*	*0.0024*	---	---	*0.3*	---	---	---	---	---
20	19-Sep-00	0.0075 [1]	0.0049	0.0047 [1]	0.0771	0.0302	0.1073	0.2	---	---	1.5	---
	25-Apr-01	0.0050	0.0051	0.0049 [1]	0.305	0.0852	0.390 [1]	0.6	---	---	14.7	---
(duplicate)	25-Apr-01	0.0081 [1]	0.0059	0.0085 [1]	0.215	0.0653	0.280	0.5	---	---	11.9	---
21	19-Sep-00	0.0034	0.0019	0.0023	0.0274	0.0070	0.0344	<0.1	---	---	---	<0.7
	25-Apr-01	0.0069 [1]	0.0040	0.0073 [1]	0.0433	0.0084	0.0517	<0.1	---	---	<0.6	---
22	19-Sep-00	0.0053 [1]	0.0011	0.0013	0.0178	0.0082	0.0260	<0.1	---	---	---	<0.7
	25-Apr-01	0.0033	<0.0004	0.0011	0.0135	0.0033	0.0168	<0.1	---	---	<1	---
23	19-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
24	19-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.7	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
25	28-Jun-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	---	(0.8)
	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	---	<0.6
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
26	7-Mar-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
27	7-Mar-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
28	8-Mar-01	0.0008	0.0022	(0.0006)	0.0168	0.0166	0.0334	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
MW9902	28-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
(duplicate)	28-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	28-Jun-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	---	66.8
	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	2.1	---
MW9903	27-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
MW9905	28-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	18-Sep-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---

Table A-4

Water Quality: Dissolved Hydrocarbon Analyses

Monitoring Station	Date (d-m-y)	Benzene (mg/L)	Toluene (mg/L)	Ethylbenzene (mg/L)	Xylene-m&p (mg/L)	Xylene-o (mg/L)	Xylenes-total (mg/L)	TPH (C_3-C_{10}) (mg/L)	TVH (C_3-C_{10}) (mg/L)	TPH (C_{11}-C_{15}) (mg/L)	TEH (C_{11}-C_{30}) (mg/L)	TEH (C_{11}-C_{60}) (mg/L)
Canadian Drinking Water Guidelines		*0.005*	*0.024*	*0.0024*	—	—	*0.3*	—	—	—	—	—
EW-1	8-Sep-00	<0.0009	<0.0009	<0.0009	<0.002	<0.0009	<0.0029	<0.1	---	---	---	2.1
	11-Sep-00	<0.0004	<0.0004	<0.0004	(0.0009)	<0.0004	(0.00090 - 0.0013)	<0.1	---	---	---	44.0
	14-Sep-00	<0.0004	(0.0006)	<0.0004	(0.0013)	0.0009	0.0022	<0.1	---	---	---	10.3
	21-Sep-00	0.0043	0.0094	0.0065 [1]	0.123	0.0383	0.1610	0.2	---	---	<0.6	---
	30-Sep-00	0.0042	0.0096	0.0068 [1]	0.106	0.0417	0.1477	---	---	---	---	---
Trip Blank	5-Jul-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	29-Aug-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	19-Sep-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	18-Oct-96	<0.0009	<0.0009	<0.0009	<0.0009	<0.0009	<0.0018	<0.02	---	<0.02	<0.2	---
	22-Nov-96	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	17-Dec-96	<0.0009	<0.0009	<0.0009	<0.0009	<0.0009	<0.0018	<0.02	---	<0.02	<0.2	---
	10-Jan-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	11-Feb-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	2-Apr-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	16-Apr-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	30-Apr-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	27-May-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.2	---
	4-Jul-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	25-Jul-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	26-Aug-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	30-Sep-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	19-Nov-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	1-Dec-97	<0.0004	(0.0007)	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	31-Dec-97	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.02	---	<0.02	<0.01	---
	4-Feb-98	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0008	<0.020	---	<0.020	<0.010	---
	22-May-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	---	---
	28-Oct-98	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.02	---
	16-Mar-99	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	11-May-99	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	---	---
	28-Jan-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	7-Apr-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
(duplicate)	7-Apr-00	<0.0005	<0.0005	<0.0005	---	---	<0.0005	---	<0.1	---	<0.05	---
	4-May-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	22-Nov-00	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---
	25-Apr-01	<0.0004	<0.0004	<0.0004	<0.0008	<0.0004	<0.0012	<0.1	---	---	<0.6	---

Table A-4
Water Quality: Dissolved Hydrocarbon Analyses

Monitoring Station	Date (d-m-y)	Benzene (mg/L)	Toluene (mg/L)	Ethylbenzene (mg/L)	Xylene-m&p (mg/L)	Xylene-o (mg/L)	Xylenes-total (mg/L)	TPH (C_3-C_{10}) (mg/L)	TVH (C_3-C_{10}) (mg/L)	TPH (C_{11}-C_{12}) (mg/L)	TEH (C_{11}-C_{30}) (mg/L)	TEH (C_{16}-C_{30}) (mg/L)
Canadian Drinking Water Guidelines		*0.005*	*0.024*	*0.0024*	—	—	*0.3*	—	—	—	—	—

NOTES:

1. — in guideline row(s) denotes no criteria for that parameter

2. — in detail row(s) denotes parameter not analyzed

3. (0.001) Single bracketed results are values below the reliable detection level, and are subject to reduced levels of confidence.

4. (1.3 - 4.5) Bracketed results with a hyphen indicate a range - typically summed fields with a mixture of non-detects and detects (values for summed data may show extra significant digits for clarity).

5. Superscript ^{1} denotes values exceeding Guidelines for Canadian Drinking Water Quality (Health Canada, 1996).

Table A-5. Data from Sampling using the Waterra Pump at Wells 3 and 8 (September 21st, 2000)

	Monitoring Well			
Compound (mg/L)	**3**	**3 (duplicate)**	**8**	**8 (duplicate)**
Benzene	0.02	0.01	<0.0004	<0.0004
Toluene	0.088	0.087	<0.0004	<0.0004
Ethylbenzene	0.014	0.015	<0.0004	<0.0004
m&p-Xylene	0.63	0.61	<0.0008	<0.0008
o-Xylene	0.187	0.179	<0.0004	<0.0004
TPH	0.9	0.9	<0.1	<0.1
C11	3.8	2.49	<0.02	<0.02
C12	5.74	3.8	<0.02	<0.02
C13	9.2	5.37	<0.02	<0.02
C14	6.61	4.74	<0.02	<0.02
C15	5.73	3.61	<0.02	<0.02
C16	2.92	1.82	<0.02	<0.02
C17	2.76	1.54	<0.02	<0.02
C18	1.7	1.21	<0.02	<0.02
C19	1.21	0.73	<0.02	<0.02
C20	0.83	0.5	<0.02	<0.02
C21	0.63	0.36	<0.02	<0.02
C22	0.26	0.14	<0.02	<0.02
C23	0.17	0.09	<0.02	<0.02
C24	0.11	0.05	<0.02	<0.02
C25	0.08	0.03	<0.02	<0.02
C26	0.06	<0.02	<0.02	<0.02
C27	0.03	<0.02	<0.02	<0.02
C28	0.04	<0.02	<0.02	<0.02
C29	<0.02	<0.02	<0.02	<0.02
C30	0.02	<0.02	<0.02	<0.02
TEH	41.9	26.5	<0.6	<0.6
Calculated Parameters				
Hardness ($CaCO_3$) (mg/L)	460	450	320	320
Ion Balance	1.07	1.07	1	1
Misc. Inorganics				
Conductivity (uS/cm)	790	771	576	578
pH	7.06	7.07	7.37	7.4
Total Dissolved Solids (mg/L)	439	429	326	325
Anions (mg/L)				
Alkalinity (PP as CaCO3)	<0.1	<0.1	<0.1	<0.1
Alkalinity (Total as CaCO3)	436	427	314	315
Bicarbonate (HCO3)	532	521	383	384
Carbonate (CO3)	<0.5	<0.5	<0.5	<0.5
Dissolved Chloride (Cl)	7.9	7.5	2.5	2.9
Hydroxide (OH)	<0.5	<0.5	<0.5	<0.5
Dissolved Sulfate (SO4)	0.4	0.3	15	13.9

Table A-5 Continued

Compound	3	3 (duplicate)	8	8 (duplicate)
Nutrients (mg/L)				
Total Ammonia (N)	0.01	<0.01	<0.01	<0.01
Nitrate plus Nitrite (N)	<0.003	<0.003	0.169	0.172
Total Phosphate (P)	0.003	<0.003	0.004	0.003
Elements (Dissolved) (mg/L)				
Aluminum (Al)	<0.001	<0.001	<0.001	<0.001
Anitmony (Sb)	<0.0002	<0.0002	<0.0002	<0.0002
Arsenic (As)	<0.005	<0.005	<0.005	<0.005
Barium (Ba)	0.207	0.207	0.135	0.14
Beryllium (Be)	<0.0002	<0.0002	<0.0002	<0.0002
Boron (B)	<0.01	<0.01	<0.01	<0.01
Cadmium (Cd)	<0.0002	<0.0002	<0.0002	<0.0002
Calcium (Ca)	121	118	86.1	85.8
Chromium (Cr)	<0.001	<0.001	<0.001	<0.001
Cobalt (Co)	0.0014	0.0016	0.0022	0.0013
Copper (Cu)	0.0013	0.0009	0.0024	0.0016
Iron (Fe)	0.04	0.03	0.03	0.02
Lead (Pb)	<0.0003	<0.0003	<0.0003	<0.0003
Lithium (Li)	0.008	0.007	0.006	0.006
Magnesium (Mg)	38.9	38.1	24.8	24.7
Manganese (Mn)	0.416	0.413	<0.004	<0.004
Molybdenum (Mo)	<0.0002	<0.0002	0.0005	0.0005
Nickel (Ni)	0.0048	0.0047	0.0039	0.0039
Phosphorus (P)	<0.1	<0.1	<0.1	<0.1
Potassium (K)	1.6	1.6	1.5	1.6
Selenium (Se)	<0.007	<0.007	<0.007	<0.007
Silicon (Si)	6.31	6.25	5.16	5.09
Silver (Ag)	<0.0001	<0.0001	<0.0001	<0.0001
Sodium (Na)	7.6	7.7	6.9	6.8
Strontium (Sr)	0.526	0.54	0.467	0.484
Sulfur (S)	0.5	0.4	5.2	5.1
Thallium (Tl)	<0.0002	<0.0002	<0.0002	<0.0002
Tin (Sn)	<0.001	<0.001	<0.001	<0.001
Titanium (Ti)	<0.001	<0.001	<0.001	<0.001
Uranium (U)	0.0007	0.0007	0.0008	0.0009
Vanadium (V)	<0.001	<0.001	<0.001	<0.001
Zinc (Zn)	0.0029	0.0054	0.0063	0.0049
Zirconium (Zr)	<0.0002	<0.0002	<0.0002	<0.0002

APPENDIX B

GROUNDWATER ANAYSES METHODOLOGIES FROM MAXXAM ANALYTICS

Maxxam
Analytics Inc.
Edmonton, Alberta, Canada T6B 2R4 Tel: (403) 468-3500 Toll-free: 1800-386-7247 Fax: (403) 466-3332 Website www.maxxam.com

Total Extractable Hydrocarbons C_8-C_{30}

This method covers the analysis of trace hydrocarbons (C_8-C_{30}) in solids and aqueous liquids. For water samples, a 1.0 liter water sample is extracted with carbon disulfide (CS_2) using heptane as a surrogate component. The extract is analysed by GCFID. For soil samples, a known amount of sample is extracted with methylene chloride/CS_2/acetone and the extract is analyzed by GCFID.

A minimum five point calibration is done with a series of n-alkane components at known concentrations. Quantification is done by summation of the area under the chromatogram in comparison with the calculated response for the n-alkane range. The method is an external standard technique. Calibrations are checked every twenty runs by a check standard which must be with 20% of the theoretical value for each n-alkane component.

Both instrument and solvent blanks are run throughout the sequence with actual samples. This procedure is limited to analytes in the range of 1 to 2000 ppm with the detection limit calculated by a program based on sample weight and volume of methylene chloride.

Methanol, other alcohols and similar solvents can cause interference with the extraction of non-polar components.

Detection Limit -1.0 ppm per component for soils

0,01 ppm per component for waters

	Soil	Water
Average surrogate recovery:-	95.5% on 100 samples	89.1% on 52 samples
Standard deviation -	14.1%	18.2%

Reference: Test Methods for Evaluating Solid Waste, SW-846; U.S. Environmental Protection Agency. Methods 3550, 8000, 3610A.
ASTM D2887-89 "Boiling Range Distribution of Petroleum Fractions by Gas Chromatography".

Total Extractable Hydrocarbons C_8-C_{80}

Samples are prepared, analysed and quantified as with the C_8 to C_{30} analysis. The key modification is the used of a stainless steel GC column capable of separating components at upper temperatures of 400°C. Again, detection is by flame ionization detector. Detection limits reflect the less sensitive nature of this technique in the C_{40} to C_{80} range.

Total Extractable Hydrocarbons (C100, Hydrocarbon Division)

Samples are extracted using carbon disulfide or methylene chloride. The surrogate added is C16 with C32 used as the internal standard. The sample extract is analyzed using gas chromatography and flame ionization detection. Sample size is 0.1 -50 gms

Polychlorinated Biphenyls in Water

rev Sept97

A known volume of sample is spiked with a known concentration of surrogate and extracted with methylene chloride. After concentrating to dryness, the sample is reconstituted to a known volume with hexane, which has been spiked with an internal standard. The sample is charred with sulfuric acid to remove and interfering organics, then analyzed by gas chromatography with an electron capture detector. Quantitation is performed by combining sample integrated areas with response factors determined by at least a five point calibration curve.

Reference Test Methods for Evaluatina Soliq Waste, SW-846; U.S. Environmental Protection Agency. Methods 3510A, 3600A, 3620A, 3650A, 3660A, 8000A, and 8080A

Purgeable Organics (Volatiles)

For volatile organic analysis (VOA) and BTEX (benzene, toluene, ethyl benzene and xylenes), a temperature programmable gas chromatograph is used to separate the analytes. Once separated, components are identified and quantified by mass spectrometry. An open split interface between the gas chromatograph and mass spectrometer eliminates the need for cryofocusing.

The mass spectrometer is capable of scanning from 40-300 amu every 1.0 sec. using 70 volts electron energy in the electron impact mode and producing a mass spectrum that meets all the tuning criteria as set out in Table 2. The iontrap mass spectrometer is used because of its inherent sensitivity over quadrapole instruments.

Calibration for all components is based on a five to seven point calibration using the internal standard method. Calibration standards are verified against two independent standards prior to use of the calibration to quantify sample results. Total purgeables are calibrated using a mixture of components. Quantification of purgeables is done on the basis of summing the area of the chromatogram, and in conjunction with the internal standard for the sample and the calibration response factor, a final result is obtained.

A complete standard mixture is analyzed to check the stability of the calibration with approximately every 15 samples analyzed. The values obtained from sample data will be compared with known concentration values. If any component is greater or less than 15% from the theoretical value, the data is discarded and the sample is reanalyzed following recalibration.

Water samples must be submitted in duplicate as one vial is used for prescreen analysis by GCFID, and the second vial is used for the definitive purge and trap analysis. A minimum of 20 g of soil is required per analysis. The sample is prescreened by extraction with methanol followed by extract analysis with GCFID. If the prescreen shows the sample is severely contaminated, the extract is analyzed by purge and trap GCMS. If the prescreen indicates the sample is relatively clean, a subsample of the original soil is analyzed by GCMS.

Typical performance characteristics are given below.

Analyte	**Average % Recovery**	**Standard Deviation**	**Concentration (ug/L)**
Benzene	100.8	1.3	12.2
Bromodichloromethane	108.9	10.0	26.7
Bromoform	97.5	8.3	40.9
Carbon Tetrachloride	87.0	17.6	109
Chlorobenzene	45.0	1.0	31.0
Chloroform	90.2	8.9	61.3
Dibromochloromethane	78.0	5.4	51.5
Dichloroethane	88.5	4.8	41.1
Ethylbenzene	93.0	9.2	115
Methylene Chloride	84.0	4.9	61.5
1,1,2,2-Tetrachloroethane	82.0	1.5	61.2
Tetrachloroethene	95.9	3.7	122
Toluene	88.0	1.0	39.8
1,1,1-Trichloroethane	82.0	3.7	60.3
Trichloroethene	145	7.3	18.1
o-Xylene	81.4	0.5	16.7
p-Xylene	90.6	1.5	47.9

Detection limit depends on the matrix of the sample, weight or volume of sample, amount of analyte found in the blank, and the dilution factor. The typical detection limit for clean waters is 0.4 ppb (w/w), while the typical detection limit for clean soils is 2 ppb (w/w).

Reference: Test Methods for Evaluating Solid Waste, SW-846; U.S. Environmental Protection Agency. Method 8260.

Chloride -(Autoanalyzer) NAQUADAT 17203L

In an automated system thiocyanate ions (SCN) are liberated from mercuric thiocyanate through sequestration of mercury by chloride ion to form un-ionized mercuric chloride. In the presence of ferric ion, the liberated SCN forms highly colored ferric thiocyanate in concentration proportional to the original chloride concentration.

Waters analysis by Technicon

Detection Limit 0.5 mg/L
Average Percent Recovery: 100.3 at 36 mg/L
Standard Deviation: 2.95 mg/L at 36 mg/L

Reference: Standard Methods for Examination of Water and Wastewater, 16th ed. American Public Health Association, Washington, DC, 1985, Method 407 D.

Methods for Chemical Analysis of Water and Wastes, U.S. Environmental Protection Agency EMSL, Cincinnati, OH 45268, March, 1979 (EPA-600/4-79-020), Method 235.2.

Sulfate- (Autoanalyzer) NAQUADAT 16306L

Sulfate ion is reacted with barium chloride and methythymol blue at pH 2.3 -3.0. The pH is then raised to 12.5 -13.0, where excess barium reacts with methythymol blue to produce a blue-colored chelate. The' mount of gray, uncomplexed methythymol blue indicates the concentration of Sulfate ion. Absorbances of excess methythymol blue is measured at 460 nm.

Waters analysis by Technicon

Detection Limit 0.5 mg/L
Average Percent Recovery: 99.5 at 42 mg/L
Standard Deviation: 2.04 mg/L at 42 mg/L

Reference: Methods for Chemical Analysis of Water and Wastes, U.S. Environmental Protection Agency, EMSL, Cincinnati, OH 45268, March, 1979 (EPA-600/4-79-020), Method 375.2.

Ion Chromatography	NAQUADAT 20112L
Calcium	NAQUADAT 12109L
Magnesium Sodium	NAQUADAT 11109L
Potassium	NAQUADAT 19109L
Ammonia	NAQUADAT 07502L

Samples are injected onto a *low* capacity, high efficiency CS10 (cation) column. Ions are separated according to their charge to mass ratio and elute at specific times from the column. Eluent is passed through a micromembrane suppressor to reduce background conductivity of eluent and to convert the ions to their acidic form which has a greater conductivity. Detection is by conductivity.

	Average Percent Recovery	Standard Deviation
Calcium	99.8 at 24.8 mg/L	39 at 24.8 mg/L
Magnesium	99.7 at 19.8 mg/ L	1.32 at 19.8 mg/L
Sodium	98.5 at 2.5 mg/L	0.56 at 2.5 mg/L
Potassium	100.6 at 0.22 mg/L	3.05 at 0.22 mg/L
Ammonia	101.5 at 0.04 mg/L	5.31 at 0.04 mg/L

Detection limits are matrix dependent.
Reference: Dionex Application Notes 2

Mercury (Water) -(Cold Vapor)
A sample aliquot is mixed with $KMnO_4$ and concentrated H_2SO_4, then digested at 90-110°C. This solution is then mixed with hydroxylamine hydrochloride and NaCl, then $SnSO_4$ and $K_2S_2O_8$ The mixture is sparged with air and the liquid removed by a gas separator. The air flow, containing Hg vapor, is then passed through an absorption cell. Absorbance is measured spectrophotometrically at 253.7 nm and compared to identically prepared standard mercury solutions.
Detection Limit: 0.05 ug/L
Average Percent Recovery: 101.5
Standard Deviation: 0.06 ug/L

Reference: Methods for Chemical Analysis of Water and Wastes, U.S. Environmental Protection Agency, EMSL, Cincinnati, OH 45268, March, 1979 (EPA-600/4-79-020), Method 245.2

Standard Methods for the Examination of Water and Wastewater, 16th ed., American Public Health Association, Washington, D.C. 1985, Method 303F.

Ammonia as Nitrogen (NAQUADA T 07555L)
Ammonia is measured by means of the Berthelot Reaction on an autoanalyzer with alkaline phenol, EDTA and NaOCl and compared to identically prepared standards. Detection Limit: 0.01 mg/L
Average Percent Recovery: 99.9%
Standard Deviation: 11.8

Reference: Standard Methods for Examination of Water and Wastewater, 16th ed., American Public Health Association, Washington, DC, 1985, Method 417C

Nitrate-Nitrite as Nitrogen* (NAQUADAT 07110L)
A sample aliquot is mixed with a disodium EDTA and passed through a column of Cd filings. A sulphanilamide solution, then a N-1-naphthylethylenediamine dihydrochloride solution are added to the sample to form an azo dye. The dye intensity is measured spectrophotometrically at 550 nm and compared with those of standard nitrate-nitrite standards.
Detection Limit: 0.003 mg/L
Average Percent Recovery: 99.5
Standard Deviation: 0.004 at 0.074 mg/L

Reference. Methods for Chemical Analysis of Water and Wastes, U.S. Environmental Protection Agency, EMSL, Cincinnati, OH 45268, March, 1979 (EPA-600/4-79-020}, Method 353.2.

Standard Methods for Examination of Water and Wastewater, 19th ed., American Public Health Association, Washington, DC, 1995, Method 4500-NO3E.

APPENDIX C

GAS CHROMATOGRAPHIC ANALYSIS OF CH_4 FOR GEOCHEMICAL EVIDENCE

CALIBRATION CURVE AND DATA

Methane Standard Curve (April 18th, 2001)

%CH4	Volume Injected (mL)	Peak Area	Partial Pressure
0.16	0.1	19983	0.0016
0.16	0.1	18924	0.0016
0.16	0.1	19287	0.0016
4	0.1	492240	0.04
4	0.1	486200	0.04
4	0.1	496890	0.04
8	0.1	966540	0.08
8	0.1	961890	0.08
8	0.1	915190	0.08

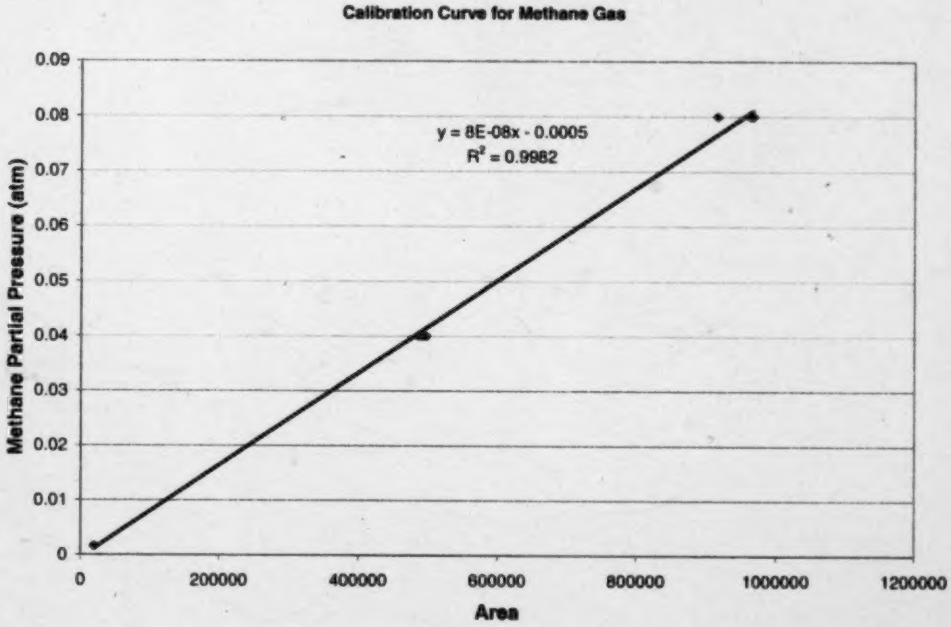


Table C-1. Dissolved Methane Concentrations

Temperature	25°C
Molecular Weight	16g/mol
Headspace Volume (Vg)	50 mL
Liquid Volume (Vw)	75 mL
Henry's Constant	41300

$$\boxed{C = MW\left[\frac{55.5}{H} + \frac{V_g}{V_w}\frac{1}{22.4}\frac{273}{(T+273)}\right]X_g}$$

Monitoring Well	Replicate Number	Peak Area	Partial Pressure = Xg y=8E-8x - 0.0005	GW Conc (mg/L)	Average Conc (mg/L)
7	1	43168	0.003	1.35	1.22
		45389	0.003	1.43	
		44074	0.003	1.39	
	2	49462	0.003	1.58	
		43656	0.003	1.37	
		47481	0.003	1.51	
	3	27431	0.002	0.78	
		27458	0.002	0.78	
		26866	0.002	0.75	
8	1	313220	0.025	11.24	10.69
		307980	0.024	11.05	
		285960	0.022	10.24	
	2	257020	0.020	9.18	
		262210	0.020	9.37	
		267940	0.021	9.58	
	3	324470	0.025	11.65	
		337270	0.026	12.12	
		326690	0.026	11.73	
19	1	233050	0.018	8.31	9.50
		232710	0.018	8.29	
		231510	0.018	8.25	
	2	306890	0.024	11.01	
		308210	0.024	11.06	
		318420	0.025	11.43	
	3	268880	0.021	9.62	
		226400	0.018	8.06	
		266000	0.021	9.51	
3	1	431870	0.034	15.59	13.28
		421630	0.033	15.21	
		418440	0.033	15.09	
	2	315250	0.025	11.32	
		356010	0.028	12.81	
		339460	0.027	12.20	
	3	344170	0.027	12.37	
		347560	0.027	12.50	
		344750	0.027	12.40	

Table C-1 Continued

Monitoring Well	Replicate Number	Peak Area	Partial Pressure = Xg y=8E-8x - 0.0005	GW Conc (mg/L)	Average Conc (mg/L)
4	1	612010	0.048	22.18	20.22
		614390	0.049	22.27	
		623390	0.049	22.60	
	2	664650	0.053	24.11	
		692180	0.055	25.12	
		685760	0.054	24.88	
	3	380220	0.030	13.69	
		388250	0.031	13.99	
		366040	0.029	13.18	
10		No methane detected			
16		No methane detected			

APPENDIX D

BART™

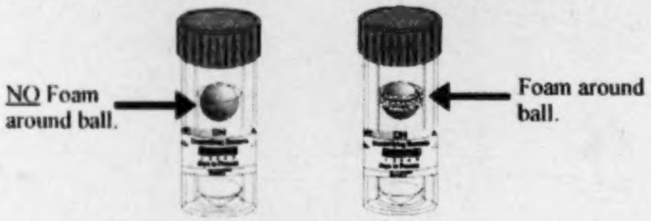
INTERPRETATION SHEETS AND RESULTS

BART™ TEST FOR DN
DENITRIFYING BACTERIA

Present/Absent - observe daily for 3 days.

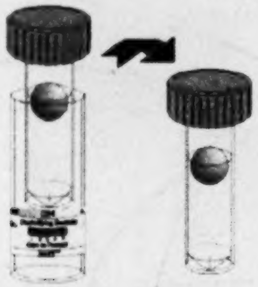
ABSENT
(Negative - Non-aggressive)

PRESENT
(Positive - Aggressive)



*Note: Refer to page bottom for approximate population

DN-BART (LAB) - Advanced test information.



1. View at the end of day 3 of test by removing the inner test vial from the outer.

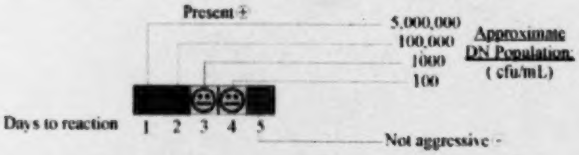
2. Observe any growths/color changes.

3. Compare with descriptions below.

Determination of Dominant Bacteria

FOAM around ball **(FO)** - Denitrifying Bacteria.

Determination of Potential DN Population - observe daily for reaction.



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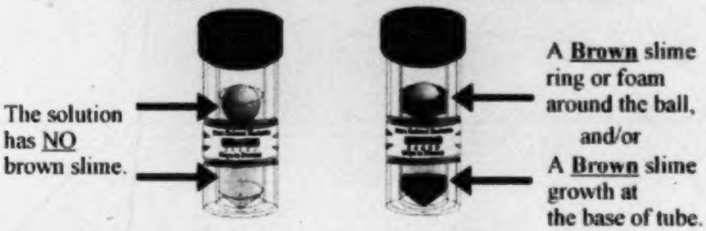
BART™ TEST FOR IRB
IRON RELATED BACTERIA

Present/Absent - observe daily for 8 days.

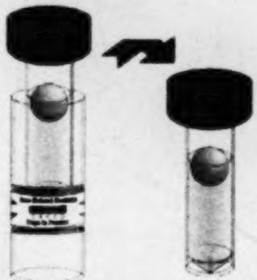
ABSENT
(Negative - Non-aggressive)

PRESENT
(Positive - Aggressive)


The solution
has NO
brown slime.
A Brown slime
ring or foam
around the ball,
and/or
A Brown slime
growth at
the base of tube.

*Note: Refer to page bottom for approximate population

IRB-BART (LAB) - Advanced test information.

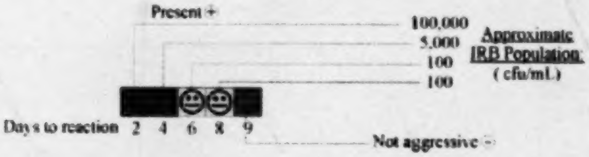


1. View at the end of day 8 of test by removing the inner test vial from the outer.
2. Observe any growths/color changes.
3. Compare with descriptions below.

Determination of Dominant Bacteria

FOAM**(FO)** around ball- Anaerobic Bacteria.
BROWN RINGS**(BR)**, GEL**(BG)**, and/or CLOUDS**(BC)** - IRB.
Solution GREEN-CLOUDY**(GC)** - Pseudomonads.
Solution RED-CLOUDY**(RC)** - Enteric Bacteria.
Solution CLOUDY**(CL)** - Heterotrophic Bacteria.
Solution BLACK**(BL)** - Pseudomonads and Enterics

Determination of Potential IRB Population - observe daily for reaction.



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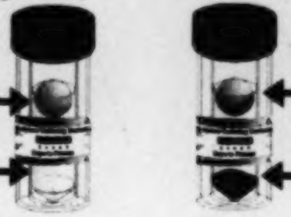
BART™ TEST FOR SRB
SULFATE REDUCING BACTERIA

Present/Absent - observe daily for 8 days.

ABSENT
(Negative - Non-aggressive)

PRESENT
(Positive - Aggressive)



The solution has NO black slime.

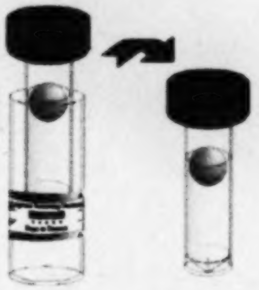
A **Black** slime ring beneath the ball,

and/or

A **Black** slime growth at the base of tube.

*Note Refer to page bottom for approximate population

SRB-BART (LAB) - Advanced test information.



1. View at end of day 8 of test by removing the inner test vial from the outer.

2. Observe any growths/color changes.

3. Compare with descriptions below.

Determination of Dominant Bacteria

BLACK only in BASE**(BB)** - Dense slime bacterial and SRB consortium.
BLACK only around BALL/TOP**(BT)** - Aerobic slime bacterial and SRB consortium.
BLACK in BASE and around BALL**(BA)** - Complex bacterial consortium with SRB present.
Solution CLOUDY - Anaerobic bacteria present.

Determination of Potential SRB Population - observe daily for reaction

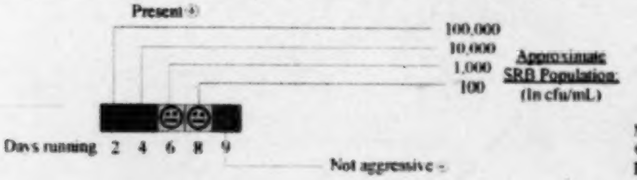


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Table D-1. Results from BART™

Monitoring Well	Temp. (°C)	BART	Lag Time	Aggressivity	Possible Population Value	Description of Reaction	Possible type of bacteria
16.1	10C	IRB	6	Medium	100	A reddish brown ring around the ball and a foam ring around the ball	Mixed anaerobic with some aerobic slime forming bacteria
16.2	10C	IRB	6	Medium	100	A reddish brown ring around the ball and a foam ring around the ball, then the liquid turned black (secondary or tertiary reaction)	Mixed anaerobic with some aerobic slime forming bacteria, the blackened liquid indicates the presence of enteric bacteria
3.1	10C	IRB	7	Medium	100	A reddish brown ring around the ball and a foam ring around the ball, then the liquid turned black (secondary or tertiary reaction)	Mixed anaerobic with some aerobic slime forming bacteria, the blackened liquid indicates the presence of enteric bacteria
3.2	10C	IRB	7	Medium	100	A reddish brown ring around the ball and a foam ring around the ball	Mixed anaerobic with some aerobic slime forming bacteria
8.1	10C	IRB	6	Medium	100	Foam at top around the ball, reddish brown ring around the ball, the tube is clear and dark red	Mixed anaerobic with some aerobic slime forming bacteria
8.2	10C	IRB	6	Medium	100	Foam at top around the ball, reddish brown ring around the ball, the tube is clear and dark red	Mixed anaerobic with some aerobic slime forming bacteria
16.1	10C	DN	5	Low	10	Foam around the ball	Denitrifying bacteria detected
16.2	10C	DN	5	Low	10	Foam around the ball	Denitrifying bacteria detected
3.1	10C	DN	7	Low	10	Foam around ball	Denitrifying bacteria detected
3.2	10C	DN	7	Low	10	Foam around ball	Denitrifying bacteria detected
8.1	10C	DN	5	Low	10	Foam around ball	Denitrifying bacteria detected
8.2	10C	DN	5	Low	10	Foam around ball	Denitrifying bacteria detected

Table D-1. Continued

Monitoring Well	Temp. (°C)	BART	Lag Time	Aggressivity	Possible Population Value	Description of Reaction	Possible type of bacteria
16.1	10C	SRB	11+	Undetected	<1	No reaction	
16.2	10C	SRB	11+	Undetected	<1	No reaction	
3.1	10C	SRB	8	Medium	100	Black around the base and black specks on the ball	Anaerobic sulfate reducing bacteria dominate
3.2	10C	SRB	8	Medium	100	Black around the base and black specks on the ball	Anaerobic sulfate reducing bacteria dominate
8.1	10C	SRB	11+	Undetected	<1	No reaction	
8.2	10C	SRB	11+	Undetected	<1	No reaction	

IRB	Iron Reducing Bacteria	16	Upstream
SRB	Sulfate Reducing Bacteria	3	Plume
DN	Denitrifying bacteria	8	Downstream

APPENDIX E

LIQUID MEDIA RECIPES FOR MICROBIAL ENUMERATION

AMENDMENT RECIPES FOR MICROBIAL ACTIVITY EXPERIMENTS

Media used for Each Group of Microorganisms

Group being Enumerated	Medium	Positive Culture Used
Total Heterotrophs	R2A liquid. Half strength	A heterotrophic bacterial culture
JP-4 degraders	Bushnell-Haas, plus 50uL of filter sterilized free product from the site	
Sulfate Reducers	Modified Butlin's broth, with nails	
Nitrate Reducers	Difco Nitrate broth, with inverted Durham vials	
Iron Reducers	B10 medium	

Liquid Media Recipes

R2A Liquid (Total Heterotrophs)

In 1.5 L of double distilled water add:
0.375g Yeast Extract
0.375g Casamino Acid
0.375g Glucose
0.375g Soluble Starch
0.225g K_2HPO_4
0.225g Sodium Pyruvate
0.375g Tryptone
0.375g Peptone
0.018g $MgSO_4$, anhydrous

Bushnell-Haas (JP-4 degraders)

In 1.5 L of double distilled water add:
0.3g $MgSO_4$
0.03g CaCl
1.5g Monopotassium Phosphate
2.5 g Dipotassium Phosphate
1.5 g Ammonium Nitrate
0.075g Ferric Chloride

Modified Butlin's (Sulfate Reducers)

In 1.5L of double distilled water add:
0.75g K_2HPO_4
1.5g NH_4Cl
3g Na_2SO_4
1.5g $MgSO_4.7H_2O$
0.105g $CaCl_2.2H_2O$
3.75 ml 60% Sodium Lactate
3g Sodium Acetate
1.5g NaCl
0.75g Sodium Benzoate
3.15g Sodium Pyruvate
1.5g Yeast Extract
0.006g $FeSO_4.7H_2O$
15ml Resazurin

Difco Nitrate Broth (Nitrate Reducers)

In 1.5L double distilled water add:
12g Nutrient Broth
1.5g Potassium Nitrate

B10 Medium (Iron Reducers)

In 1.5L of double distilled water:
1.2g K_2HPO_4
0.3g KH_2PO_4
0.3g $MgSO_4.7H_2O$
0.3g NaCl
1.5ml 0.1% $MnSO_4$
1.5ml 0.1% $NaSO_4$
15 ml $CaSO_4$ (saturated)
7.5g Yeast Extract
7.5g Bacto peptone
7.05g $FePO_4$
3.75g Agar

Westlake N, P (50X) Nutrient Stock (200 mL)

5g K_2HPO_4
10g NH_4Cl
20g KNO_3
200 ml double distilled water

Amendment Recipes

Sulfate Amendment (3mM)

3g Na_2SO_4
100 mL double distilled water

Nitrate Amendment (3mM)

4.62 g KNO_3
100 mL double distilled water

Resazurin (0.1 g/L) and Bicarbonate (100 g/L)

600 mL Resazurin (0.1g/L)
60 g Na_2CO_3

APPENDIX F

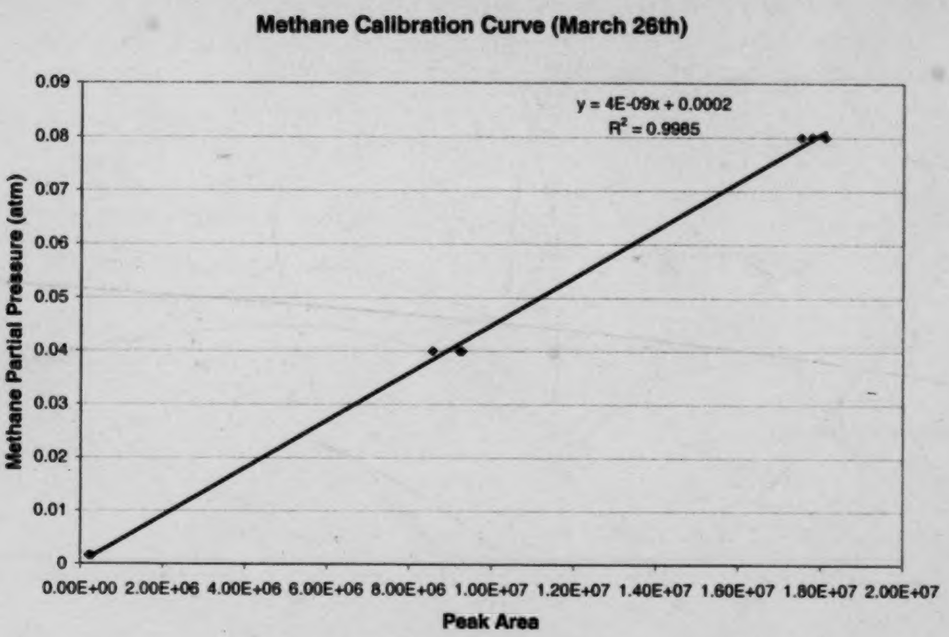
DESCRIPTION OF LIQUID SCINTILLATION DETECTOR

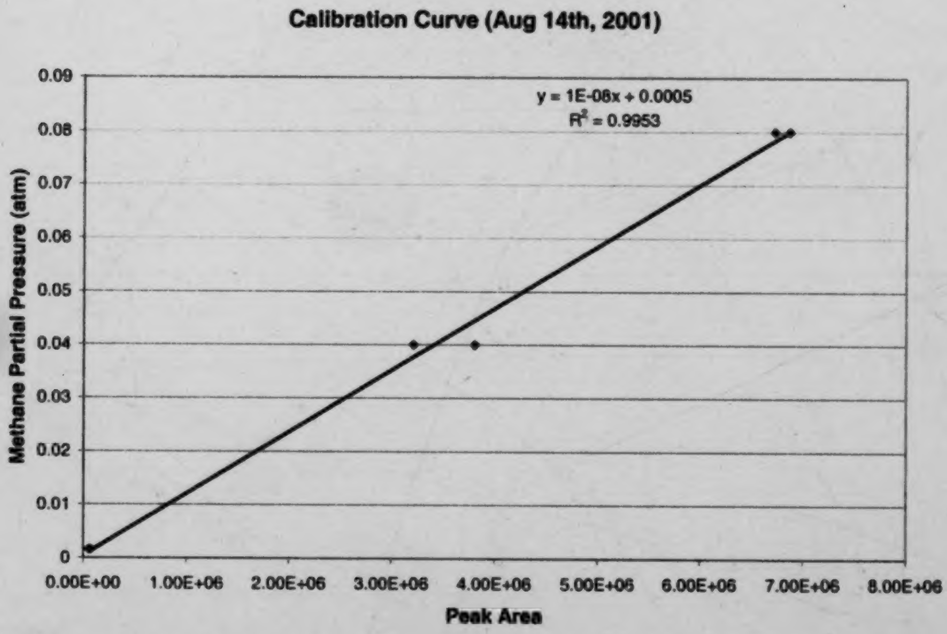
Liquid Scintillation Detector

Scintillation is defined as a process where energy deposited by ionizing radiation is absorbed and converted to light photons. There are a number of different types of scintillators including organic crystal scintillators, organic liquid scintillators or solid scintillators, inorganic crystal scintillators, and noble gas scintillators (University of Calgary, 1996). This study uses a liquid scintillation counter. Measuring radioactivity in a liquid scintillation counter involves dissolving a radioactive sample in a chemical solution called scintillation fluid or cocktail (fluor) (University of Calgary, 1996). When alpha or beta radiation is absorbed in the fluor, fluorescent light is emitted. The counter detects the fluorescent pulses using a photomultiplier tube (PMT), which converts the emitted light to electrical signals. The sample is quantified by relating the electrical signals to absorbed energy (University of Calgary, 1996). The number of electrical impulses from the radioactive sample are displayed as cpm or dpm by the counter (University of Alberta, 1996). The liquid scintillation counter is used to count radionuclides that decay by alpha and beta particle emission, including H-3, C-14, and P-32. It can also be used to measure x-rays and gamma rays and has a high counting efficiency for most radionuclides (University of Calgary, 1996; University of Alberta, 1996).

APPENDIX G

GAS CHROMATOGRAPHIC ANALYSIS OF CH_4 FOR MICROBIAL EVIDENCE





The parameters on the GC changed between May 29th and August 8th, consequently the calibration curve also changed.

Dissolved Methane Calculations

The same equation is used as in Appendix C

Temp	25	°C
MW	16	g/mol
Headspace Vol	50	mL
Liquid Volume	75	mL
Henry's Constant	41300	

Table G-1. Dissolved Methane Concentrations (March 26th, 2001)

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=4E-9x + 0.0002	GW Conc (mg/L)	Av Conc (mg/L)
Acetate	A	No	3165392	0.013	5.89	6.44
Acetate	A	Yes	3181813	0.013	5.92	
Acetate	B	No	2767752	0.011	5.16	
Acetate	B	Yes	2926430	0.012	5.45	
Acetate	C	No	3852294	0.016	7.14	
Acetate	C	Yes	4910320	0.020	9.08	
Negative	Acetate	No	3632459	0.015	6.74	6.48
Negative	Acetate	No	3652176	0.015	6.78	
Negative	Acetate	Yes	3190530	0.013	5.93	

Table G-2. Dissolved Methane Calculations (April 18th, 2001)

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=4E-9x + 0.0002	GW Conc (mg/L)	Av Conc (mg/L)
Negative Control		no	4363651	0.018	8.08	7.91
Negative Control	Dodecane	no	4265389	0.017	7.90	
Negative Control	Dodecane	yes	4174126	0.017	7.73	
Unamended 10C	A	no	4177280	0.017	7.74	7.14
Unamended 10C	A	no	4107677	0.017	7.61	
Unamended 10C	A	yes	4146234	0.017	7.68	
Unamended 10C	B	yes	3956746	0.016	7.34	
Unamended 10C	B	no	3432992	0.014	6.38	
Unamended 10C	B	yes	3647296	0.015	6.77	
Unamended 10C	C	no	3587437	0.015	6.66	
Unamended 10C	C	yes	3750530	0.015	6.96	
Unamended 10C	C	yes	3595478	0.015	6.67	
Unamended 23C	A	no	10208104	0.041	18.78	14.10
Unamended 23C	A	yes	9576896	0.039	17.63	
Unamended 23C	B	no	9344390	0.038	17.20	
Unamended 23C	B	yes	8243683	0.033	15.19	
Unamended 23C	C	no	4450874	0.018	8.24	
Unamended 23C	C	yes	4085288	0.017	7.57	
Toluene	A	no	3874000	0.016	7.18	7.64
Toluene	A	yes	3893100	0.016	7.22	
Toluene	B	no	4341900	0.018	8.04	
Toluene	B	no	4254300	0.017	7.88	
Toluene	C	no	4197900	0.017	7.78	

Table G-2. Continued

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=4E-9x + 0.0002	GW Conc (mg/L)	Av Conc (mg/L)
Toluene	C	yes	4186300	0.017	7.76	
Acetate	A	no	4185200	0.017	7.75	12.31
Acetate	A	yes	4102600	0.017	7.60	
Acetate	B	no	9400600	0.038	17.30	
Acetate	B	yes	8963100	0.036	16.50	
Acetate	C	no	4254700	0.017	7.88	
Acetate	C	yes	9133600	0.037	16.81	

Table G-3. Dissolved Methane Calculations (May 11th, 2001)

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=4E-9x + 0.0002	GW Conc (mg/L)	Av Conc (mg/L)
Acetate	A	no	4284339	0.017	7.94	6.56
Acetate	A	yes	2705598	0.011	5.05	
Acetate	B	no	3554082	0.014	6.60	
Acetate	B	yes	3403648	0.014	6.32	
Acetate	C	no	4108856	0.017	7.61	
Acetate	C	yes	3125925	0.013	5.82	
Sulfate	A	no	3685200	0.015	6.84	7.00
Sulfate	A	yes	3742674	0.015	6.94	
Sulfate	B	no	3594306	0.015	6.67	
Sulfate	B	yes	3555488	0.014	6.60	
Sulfate	C	no	3959922	0.016	7.34	
Sulfate	C	yes	4100754	0.017	7.60	

Table G-3. Continued

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=4E-9x + 0.0002	GW Conc (mg/L)	Av Conc (mg/L)
Negative Control	Dodecane	no	4843104	0.020	8.96	8.91
Negative Control	Dodecane	yes	4785821	0.019	8.85	
Nitrate	A	no	4524448	0.018	8.38	7.80
Nitrate	A	no	4340426	0.018	8.04	
Nitrate	A	yes	4272352	0.017	7.91	
Nitrate	B	no	4249136	0.017	7.87	
Nitrate	B	yes	3578901	0.015	6.64	
Nitrate	B	yes	4151256	0.017	7.69	
Nitrate	C	no	4197485	0.017	7.78	
Nitrate	C	yes	4349421	0.018	8.06	
Nutrient	A	yes	4155050	0.017	7.70	7.92
Nutrient	A	yes	4287178	0.017	7.94	
Nutrient	B	yes	4134966	0.017	7.66	
Nutrient	B	yes	4366867	0.018	8.09	
Nutrient	C	no	4562077	0.018	8.44	
Nutrient	C	yes	4259725	0.017	7.89	
Nutrient	C	yes	4180398	0.017	7.75	
Unamended 10C	A	no	3974848	0.016	7.37	7.58
Unamended 10C	A	yes	4262026	0.017	7.90	
Unamended 10C	B	no	4227206	0.017	7.83	
Unamended 10C	B	yes	4488141	0.018	8.31	
Unamended 10C	C	no	3761302	0.015	6.98	
Unamended 10C	C	yes	3817397	0.015	7.08	
Unamended 23C	A	no	10122840	0.041	18.63	

Table G-3. Continued

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=4E-9x + 0.0002	GW Conc (mg/L)	Av Conc (mg/L)
Unamended 23C	A	yes	10021616	0.040	18.44	13.17
Unamended 23C	B	no	4457674	0.018	8.25	
Unamended 23C	B	yes	4538835	0.018	8.40	
Unamended 23C	C	no	6833123	0.028	12.60	
Unamended 23C	C	yes	6874512	0.028	12.68	
Toluene	A	no	4358173	0.018	8.07	7.80
Toluene	A	yes	4550400	0.018	8.42	
Toluene	B	no	3982394	0.016	7.38	
Toluene	B	yes	4344074	0.018	8.05	
Toluene	C	no	4092146	0.017	7.58	
Toluene	C	yes	3935210	0.016	7.30	
Negative Control	Toluene	no	3566245	0.014	6.62	5.32
Negative Control	Toluene	yes	2144378	0.009	4.02	

Table G-4. Dissolved Methane Calculations (May 29th, 2001)

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=4E-9x + 0.0002	GW Conc (mg/L)	Av Conc (mg/L)
Acetate	A	yes	3174597	0.013	5.90	6.34
Acetate	A	yes	2998413	0.012	5.58	
Acetate	B	yes	3169634	0.013	5.90	
Acetate	B	yes	3463866	0.014	6.43	
Acetate	C	yes	3883584	0.016	7.20	
Acetate	C	yes	3800930	0.015	7.05	

Table G-4. Continued

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=4E-9x + 0.0002	GW Conc (mg/L)	Av Conc (mg/L)
Sulfate	A	yes	4937834	0.020	9.13	6.75
Sulfate	A	yes	4629856	0.019	8.57	
Sulfate	B	yes	2841232	0.012	5.29	
Sulfate	B	yes	2850656	0.012	5.31	
Sulfate	C	yes	3358562	0.014	6.24	
Sulfate	C	yes	3205080	0.013	5.96	
Negative Control	Dodecane	yes	3966490	0.016	7.35	7.31
Negative Control	Dodecane	yes	3874221	0.016	7.19	
Nitrate	A	yes	4119904	0.017	7.63	7.07
Nitrate	A	yes	4018165	0.016	7.45	
Nitrate	B	yes	3726706	0.015	6.92	
Nitrate	B	yes	3612016	0.015	6.71	
Nitrate	C	yes	3733584	0.015	6.93	
Nitrate	C	yes	3661370	0.015	6.80	
Nutrient	A	yes	4140190	0.017	7.67	7.40
Nutrient	A	yes	3987421	0.016	7.39	
Nutrient	B	yes	3841032	0.016	7.12	
Nutrient	B	yes	3796162	0.015	7.04	
Nutrient	C	yes	4057488	0.016	7.52	
Nutrient	C	yes	4142744	0.017	7.68	
Unamended 10C	A	yes	3587072	0.015	6.66	5.51
Unamended 10C	A	yes	3695912	0.015	6.86	
Unamended 10C	B	yes	2494581	0.010	4.66	
Unamended 10C	B	yes	2525357	0.010	4.72	

Table G-4. Continued

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=4E-9x + 0.0002	GW Conc (mg/L)	Av Conc (mg/L)
Unamended 10C	C	yes	2684722	0.011	5.01	
Unamended 10C	C	yes	2758798	0.011	5.14	
Unamended 23C	A	yes	7320941	0.029	13.50	11.11
Unamended 23C	A	yes	7829363	0.032	14.43	
Unamended 23C	B	yes	3905910	0.016	7.24	
Unamended 23C	B	yes	3894358	0.016	7.22	
Unamended 23C	C	yes	6630445	0.027	12.23	
Unamended 23C	C	yes	6520970	0.026	12.03	
Toluene	A	yes	3753368	0.015	6.96	6.68
Toluene	A	yes	3829072	0.016	7.10	
Toluene	B	yes	3285829	0.013	6.11	
Toluene	B	yes	3650704	0.015	6.78	
Toluene	C	yes	3516378	0.014	6.53	
Toluene	C	yes	3551154	0.014	6.59	

Table G-5. Dissolved Methane Calculations (August 8th, 2001)

1 = free product and extra dodecane
2 = free-product only

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=1E-8x + 0.0005	GW Conc (mg/L)	Av Conc (mg/L)
Acetate	10A	Yes	2010890	0.02	9.43	9.61
	10A	Yes	1998538	0.02	9.38	
	10B	Yes	2089051	0.02	9.79	
	10B	Yes	1887590	0.02	8.87	
	10C	Yes	2248925	0.02	10.52	

Table G-5 Continued

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=1E-8x + 0.0005	GW Conc (mg/L)	Av Conc (mg/L)
Acetate	10C	Yes	2061641	0.02	9.67	
Acetate	Negative	Yes	1915552	0.02	9.00	9.22
	Negative	Yes	2011047	0.02	9.43	
Sulfate	1A	Yes	742863	0.01	3.63	3.18
	1A	Yes	709536	0.01	3.48	
	1B	Yes	570413	0.01	2.84	
	1B	Yes	556559	0.01	2.78	
	2A	Yes	887583	0.01	4.29	3.94
	2A	Yes	876674	0.01	4.24	
	2B	Yes	743215	0.01	3.63	
	2B	yes	736545	0.01	3.60	
Dodecane (negative)	1	yes	344439	0.00	1.81	
Nitrate	1A	yes	527841	0.01	2.65	2.85
	1B	yes	617509	0.01	3.06	
	2A	yes	677912	0.01	3.33	3.27
	2B	yes	651541	0.01	3.21	
Nutrient	1A	yes	781174	0.01	3.80	3.76
	1B	yes	760371	0.01	3.71	
	2A	yes	950780	0.01	4.58	4.79
	2B	yes	1041809	0.01	5.00	
Unamended 10C	1A	yes	436618	0.00	2.23	2.51
	1A	yes	422660	0.00	2.16	

Table G-5 Continued

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=1E-8x + 0.0005	GW Conc (mg/L)	Av Conc (mg/L)
Unamended 10C	1B	yes	574327	0.01	2.86	
	1B	yes	561397	0.01	2.80	
	2A	yes	400934	0.00	2.06	1.32
	2A	yes	383377	0.00	1.98	
	2B	yes	98632	0.00	0.68	
	2B	yes	71990	0.00	0.56	
Unamended 23C	1A	yes	689473	0.01	3.38	1.84
	1A	yes	591007	0.01	2.93	
	1B	yes	56114	0.00	0.49	
	1B	yes	69591	0.00	0.55	
	2A	yes	746184	0.01	3.64	3.23
	2A	yes	740987	0.01	3.62	
	2B	yes	569040	0.01	2.83	
	2B	yes	567640	0.01	2.83	
Toluene	A	yes	2044987	0.02	9.59	9.40
	A	yes	1960536	0.02	9.20	
	B (f-p)	yes	39622	0.00	0.41	2.31
	B (f-p)	yes	38898	0.00	0.41	
	C (f-p)	yes	898092	0.01	4.34	
	C (f-p)	yes	839905	0.01	4.07	

Table G-6. Dissolved Methane Calculations (September 24th, 2001)

All dodecane samples have extra free-product and dodecane radiolabel
Toluene samples have extra free-product

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=1E-8x + 0.0005	GW Conc (mg/L)	Av Conc
Acetate	A	yes	1773294	0.018	8.35	7.79
	A	yes	1698315	0.017	8.00	
	B	yes	1421329	0.015	6.73	
	B	yes	1378874	0.014	6.54	
	C	yes	1858164	0.019	8.73	
	C	yes	1783670	0.018	8.39	
Acetate	Negative	yes	2019891	0.021	9.47	9.43
		yes	2000073	0.021	9.38	
Sulfate	A	yes	420273	0.005	2.15	3.17
	A	yes	392051	0.004	2.02	
	B	yes	880478	0.009	4.26	
	B	yes	891517	0.009	4.31	
	C	yes	637919	0.007	3.15	
	C	yes	634260	0.007	3.13	
Dodecane (negative)			841843	0.009	4.08	
Nitrate	A	yes	658103	0.007	3.24	2.57
	A	yes	624455	0.007	3.09	
	B	yes	362153	0.004	1.89	
	B	yes	329835	0.004	1.74	
	C	yes	586444	0.006	2.91	
	C	yes	504956	0.006	2.54	

Table G-6. Continued

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=1E-8x + 0.0005	GW Conc (mg/L)	Av Conc
Nutrient	A	yes	675784	0.007	3.32	2.85
	A	yes	654376	0.007	3.22	
	B	yes	302481	0.004	1.61	
	B	yes	305299	0.004	1.63	
	C	yes	759901	0.008	3.71	
	C	yes	744342	0.008	3.64	
Unamended 10C	A	yes	289334	0.003	1.55	1.78
	A	yes	254010	0.003	1.39	
	B	yes	337023	0.004	1.77	
	B	yes	380587	0.004	1.97	
	C	yes	387014	0.004	2.00	
	C	Yes	384472	0.004	1.99	
Unamended 23C	A	Yes	1040329	0.011	4.99	4.86
	A	Yes	1009680	0.011	4.85	
	B	Yes	1701432	0.018	8.02	
	B	Yes	1688534	0.017	7.96	
	C	Yes	343844	0.004	1.80	
	C	Yes	292603	0.003	1.57	
Toluene	A	Yes	1055559	0.011	5.06	4.13
	A	Yes	1091273	0.011	5.22	
	B	Yes	788831	0.008	3.84	
	B	Yes	815045	0.009	3.96	
	C	Yes	681793	0.007	3.35	
	C	Yes	687485	0.007	3.38	

Table G-7. Dissolved Methane Calculations (September 29th, 2001)

All samples have extra free-product
All samples were additionally spiked with extra free-product and dodecane
All samples were incubated inverted

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=1E-8x + 0.0005	GW Conc (mg/L)	Av Conc (mg/L)
Sulfate	A	Yes	0	0.0005	0.23	0.23
	A	yes	0	0.0005	0.23	
	B	yes	0	0.0005	0.23	
	B	yes	0	0.0005	0.23	
	C	yes	0	0.0005	0.23	
	C	yes	0	0.0005	0.23	
Dodecane (negative)			0	0.0005	0.23	
			0	0.0005	0.23	
Nitrate	A	yes	0	0.0005	0.23	0.23
	A	yes	0	0.0005	0.23	
	B	yes	0	0.0005	0.23	
	B	yes	0	0.0005	0.23	
	C	yes	0	0.0005	0.23	
	C	yes	0	0.0005	0.23	
Nutrient	A	yes	248933	0.0029893	1.37	2.22
	A	yes	256079	0.0030608	1.40	
	B	yes	1068366	0.0111837	5.12	
	B	yes	1039719	0.0108972	4.99	
	C	yes	0	0.0005	0.23	
	C	yes	0	0.0005	0.23	

Table G-7. Continued

Sample	Type	Acid?	Peak Area	Partial Pressure = Xg y=1E-8x + 0.0005	GW Conc (mg/L)	Av Conc (mg/L)
Unamended 10C	A	yes	0	0.0005	0.23	0.23
	A	yes	0	0.0005	0.23	
	B	yes	0	0.0005	0.23	
	B	yes	0	0.0005	0.23	
	C	yes	0	0.0005	0.23	
	C	yes	0	0.0005	0.23	
Unamended 23C	A	yes	0	0.0005	0.23	0.23
	A	yes	0	0.0005	0.23	
	B	yes	0	0.0005	0.23	
	B	yes	0	0.0005	0.23	
	C	yes	0	0.0005	0.23	
	C	yes	0	0.0005	0.23	

APPENDIX H

DEPARTMENT OF LIMNOLOGY, UNIVERSITY OF ALBERTA

CHEMICAL ANALYSIS METHODOLOGY AND COMPLETE RESULTS OF NITRATE AND SULFATE ANALYSES

Nitrate and Sulfate Methodology

For sulfate and nitrate analysis, EPA method 300.1 (determination of inorganic anions in drinking water by ion chromatography) was used. Briefly, a Dionex DX-600 ion chromatograph was used. The system contained a GS50 gradient pump, an AS50 chromatography compartment, an AS50 autosampler with a 100 µl loop, and a CD25 suppressed conductivity detector with an ASRS-Ultra-4mm anion self-generating suppresser. A Dionex AS9-HC 4 mm column was used for separation the anions. To protect the analytical column, an AS9-HC 4mm guard column was used. The mobile phase contained 9 mM of Na_2CO_3. Isocratic elution was performed under a flow rate of 1.2 mL/min. Dionex PeakNet 6 chromatography software was used for data acquisition and analysis. All the quality control procedures suggested in method 300.1 were followed during the sample analyses. QC sample was certified standards. The method performance in Limnology Service Unit, Department of Biological Sciences, University of Alberta reached all the criteria described in EPA method 300.1.

Chemical Analyses for Sulfate and Nitrate

Original Amendments

Nitrate = 303 mg/L

Sulfate = 426 mg/L

Table H-1. Nitrate Concentrations (mg/L) from 125 mL Microcosms

Nitrate (mg/L)	01-Dec-00	01-Mar-01	16-May-01	20-Sep-01
Negative	0.00	0.00	0.00	0.00
Sulfate A	0.00	0.1	0.00	0.00
Sulfate B	0.00	0.00	0.00	0.00
Sulfate C	0.00	0.00	0.00	0.00
Nitrate A	138.81	123.72	144.85	110.8
Nitrate B	190.56	138.94	126.28	122.5
Nitrate C	180.13	131.94	117.2	143.8
Unamended A (10°C)	0.00	0.00	0.00	0.00
Unamended B (10°C)	0.00	0.00	0.00	0.00
Unamended C (10°C)	0.00	0.00	0.00	0.00
Unamended A (20°C)	0.00	0.00	0.00	0.00
Unamended B (20°C)	0.00	0.00	0.00	0.00
Unamended C (20°C)	0.00	0.00	0.00	0.00
Nutrient A	138.77	121.12	123.72	104.2
Nutrient B	102.47	129.35	146.8	102.9
Nutrient C	129.74	140.06	117.58	115.1

Table H-2. Sulfate Concentrations (mg/L) from 125 mL Microcosms

Sulfate (mg/L)	01-Dec-00	01-Mar-01	16-May-01	20-Sep-01
Negative	0.31	0.42	0.53	0.00
Sulfate A	342.18	328.73	330.93	313.90
Sulfate B	333.99	334.3	332.92	325.90
Sulfate C	336.14	343.96	329.08	326.30
Nitrate A	0.84	0.62	0.72	0.00
Nitrate B	0.22	0.6	0.65	0.00
Nitrate C	0.49	0.54	0.63	0.00
Unamended A (10°C)	0.46	0.09	0.15	0.00
Unamended B (10°C)	0.53	0.13	0.25	0.00
Unamended C (10°C)	0.34	0.46	0.16	0.00
Unamended A (20°C)	0.52	0.08	0.00	0.00
Unamended B (20°C)	0.55	0.07	0.00	0.00
Unamended C (20°C)	0.50	0.03	0.16	0.00
Nutrient A	0.36	0.52	0.56	0.46
Nutrient B	0.39	0.56	0.64	0.42
Nutrient C	0.41	0.59	0.8	0.6

APPENDIX I

ΔH^0 AND ΔG^0 VALUES FROM THERMODYNAMIC TABLES

CALCULATIONS TO CHECK VALUES OF ΔG^0

SAMPLE CALCULATIONS TO DETERMINE ΔG AT 10°C

CALCULATED ΔH^0 VALUES

ΔH^0 and ΔG^0 Values from Thermodynamic Tables

Table I-1. Values of ΔH_f^0 and ΔG_f^0 (Stumm and Morgan, 1981; Dean, 1999)

Compound	State	ΔH_f^0 (kJ/mol)	ΔG_f^0 (kJ/mol)
Benzene	Liquid	49.0	124.4
Ethylbenzene	Liquid	-12.3	119.6
Toluene	Liquid	12.4	113.8
1,2 Xylene	Liquid	-24.4	110.3
1,3 Xylene	Liquid	-25.4	107.7
1,4 Xylene	Liquid	-24.4	110.1
CO_2	Gas	-393.5	-394.4
H_2O	Liquid	-285.8	-237.2
H^+	Aqueous	0	0
Mn(II)	Aqueous	-220.7	-228.0
MnO_2	Aqueous	-520.0	-465.1
N_2	Gas	0	0
NO_3^-	Aqueous	-207.3	-111.3
O_2	Gas	0	0
SO_4^{2-}	Aqueous	-909.2	-744.6
H_2S	Aqueous	-39.8	-27.9
CH_4	Gas	-74.8	-50.8
Dodecane	Liquid	-226.2	137.9

Calculations to check values of ΔG^0

Values of ΔG^0 were obtained from Wiedemeier et al. (1995). To ensure that the correct phase of each compound was being used for calculation of ΔH^0, ΔG^0 was calculated using values from thermodynamic tables (Stumm and Morgan, 1981; Dean, 1999). The following equation is the same as Equation 30. It is used to calculate ΔG^0 at STP using ΔG_f^0 of each compound.

$$\Delta G^0 = \sum_{products} n\Delta G_f^0 - \sum_{reactants} n\Delta G_f^0$$

The following example calculates ΔG^0 for benzene with oxygen as an electron acceptor. The equation for degradation of benzene oxidation is:

$$C_6H_6 + 7.5O_2 \rightarrow 6CO_2 + 3H_2O$$

Values of ΔG_f^0 were obtained from Table G-1.

$\Delta G^0 = \Sigma(124.4) - \Sigma\ (6*-394.4)+(3*-237.2)$

$\Delta G^0 = 3202.2$ kJ/mol

The following table summarizes the ΔG^0 calculated for benzene, toluene, ethylbenzene, xylenes and dodecane under aerobic, denitrifying, Mn(IV) reducing, sulfate-reducing and methanogenic conditions.

Table I-2. Calculated and literature ΔG^0 values (Wiedemeier et al, 1995a).

Benzene	ΔG_f^0 (r)	ΔG_f^0 (p)	ΔG^0 (calculated)	ΔG^0 (literature)
Oxidation	124.4	-3077.8	-3202.2	-3202.0
Denitrification	-543.4	-3789.3	-3245.9	-3245.0
Manganese Reduction	-6852.1	-10055.5	-3203.4	-3202.0
Sulfate Reduction	-2667.9	-3182.3	-514.4	-514.3
Methanogenesis	-942.9	-1077.6	-134.7	-135.6

Table I-2 Continued

Toluene	**ΔG_f^0 (r)**	**ΔG_f^0 (p)**	**ΔG^0 (calculated)**	**ΔG^0 (literature)**
Oxidation	113.8	-3709.3	-3823.1	-3823.0
Denitrification	-687.6	-4563.2	-3875.6	-3875.0
Manganese Reduction	-8258.0	-12082.6	-3824.6	-3824.0
Sulfate Reduction	-3236.9	-3834.7	-597.8	-597.7
Methanogenesis	-1072.1	-1214.3	-142.2	-142.6

Ethylbenzene	**ΔG_f^0 (r)**	**ΔG_f^0 (p)**	**ΔG^0 (calculated)**	**ΔG^0 (literature)**
Oxidation	119.6	-4340.9	-4460.5	-4461.0
Denitrification	-815.3	-5337.0	-4521.7	-4522.0
Manganese Reduction	-10112.6	-14812.0	-4699.4	-4461.0
Sulfate Reduction	-3789.6	-4487.2	-697.6	-697.7
Methanogenesis	-1184.9	-1351.0	-166.1	-166.7

1,3 Xylene	**ΔG_f^0 (r)**	**ΔG_f^0 (p)**	**ΔG^0 (calculated)**	**ΔG^0 (literature)**
Oxidation	107.7	-4340.9	-4448.6	-4448.0
Denitrification	-827.2	-5337.0	-4509.8	-4509.0
Manganese Reduction	-10124.5	-14812.0	-4687.5	-4449.0
Sulfate Reduction	-3801.5	-4487.2	-685.7	-685.6
Methanogenesis	-1196.8	-1351.0	-154.2	-154.6

1,2 Xylene	**ΔG_f^0 (r)**	**ΔG_f^0 (p)**	**ΔG^0 (calculated)**	**ΔG^0 (literature)**
Oxidation	110.3	-4340.9	-4451.2	-
Denitrification	-824.6	-5337.0	-4512.4	-
Manganese Reduction	-10121.9	-14812.0	-4690.1	-
Sulfate Reduction	-3798.9	-4487.2	-688.3	-
Methanogenesis	-1194.2	-1351.0	-156.8	-

Table I-2. Continued

1,4 Xylene	**ΔG_f^0 (r)**	**ΔG_f^0 (p)**	**ΔG^0 (calculated)**	**ΔG^0 (literature)**
Oxidation	110.1	-4340.9	-4451.0	-
Denitrification	-824.8	-5337.0	-4512.2	-
Manganese Reduction	-10122.1	-14812.0	-4689.9	-
Sulfate Reduction	-3799.1	-4487.2	-688.1	-
Methanogenesis	-1194.4	-1351.0	-156.6	-

Dodecane	**ΔG_f^0 (r)**	**ΔG_f^0 (p)**	**ΔG^0 (calculated)**	**ΔG^0 (literature)**
Oxidation	137.9	-7815.8	-7953.7	-
Denitrification	-1509.3	-9570.9	-8061.6	-
Manganese Reduction	-17070.8	-25027.4	-7956.6	-
Sulfate Reduction	-6749.7	-7288.7	-539.0	-
Methanogenesis	-1166.6	-1554.0	-387.4	-

NB: Blank spaces under ΔG^0 (literature) indicate that these values were not found in the literature

Sample Calculation to Determine ΔG values at 10°C

ΔG at 10°C is determined using the shortcut van't Hoff equation described in equation 33.

$$\Delta G_{T_2} = -\Delta H^0 * T_2\left(\frac{1}{T_2}+\frac{1}{T_1}\right)+\frac{\Delta G^0 * T_2}{T_1}$$

ΔH^0 is determined using tables of thermodynamics (Table G-1) and equation 32:

$$\Delta H^0 = \sum_{products} n\Delta H_f^0 - \sum_{reactants} nH_f^0$$

The following example determines ΔG at 10°C for benzene with oxygen as an electron acceptor. Using the reaction for degradation of benzene and ΔH^0 values from Table G-1, ΔH^0 was determined as follows:

$\Delta H^0 = = \Sigma(49) - \Sigma\ (6*-393.5)+(3*-285.8)$

$\Delta H^0 = -3267.5$ kJ/mol

This value is used in equation 25, T_1 is 298K, and T_2 is 283K.

$\Delta G_{T2} = -(-3267.5\text{kJ/mol})*283\text{K}(1/283\text{K} +1/298\text{K})+ (-3202\text{kJ/mol}*283\text{K})/298\text{K}$

$\Delta G_{T2} = -2867.6$ kJ/mol

Calculated ΔH^0 values

Table I-3. Calculated ΔH^0 values using equation 32 and ΔH_f^0 from Table G-1

Benzene	**ΔH_f (r)**	**ΔH_f (p)**	**ΔH^0**
Oxidation	49.0	-3218.5	-3267.5
Denitrification	-1194.8	-4076.0	-2881.2
Manganese Reduction	-7751.0	-10816.4	-3065.4
Sulfate Reduction	-3360.5	-3367.6	-7.1
Methanogenesis	-379.7	-1165.9	-786.1

Toluene	**ΔH_f (r)**	**ΔH_f (p)**	**ΔH^0**
Oxidation	12.4	-3897.8	-3910.2
Denitrification	-1480.2	-4926.8	-3446.6
Manganese Reduction	-9347.6	-13015.4	-3667.8
Sulfate Reduction	-4079.0	-4076.7	2.3
Methanogenesis	-1416.8	-1320.4	96.4

Ethylbenzene	**ΔH_f (r)**	**ΔH_f (p)**	**ΔH^0**
Oxidation	-12.3	-4577.2	-4564.9
Denitrification	-1753.6	-5777.6	-4024.0
Manganese Reduction	-11452.3	-16006.6	-4554.3
Sulfate Reduction	-4785.6	-4785.8	-0.2
Methanogenesis	-1584.4	-1474.8	109.5

1, 3 Xylene	**ΔH_f (r)**	**ΔH_f (p)**	**ΔH^0**
Oxidation	-25.4	-4577.2	-4551.8
Denitrification	-1766.7	-5777.6	-4010.9
Manganese Reduction	-11465.4	-16006.6	-4541.2
Sulfate Reduction	-4798.7	-4785.8	12.9
Methanogenesis	-1597.5	-1474.8	122.6

1, 2 Xylene	**ΔH_f (r)**	**ΔH_f (p)**	**ΔH^0**
Oxidation	-24.4	-4577.2	-4552.8
Denitrification	-1765.7	-5777.6	-4011.9
Manganese Reduction	-11464.4	-16006.6	-4542.2
Sulfate Reduction	-4797.7	-4785.8	11.9
Methanogenesis	-1596.5	-1474.8	121.6

Table I-3. Continued

1, 4 Xylene	**ΔH_f (r)**	**ΔH_f (p)**	**ΔH^0**
Oxidation	-24.4	-4577.2	-4552.8
Denitrification	-1765.7	-5777.6	-4011.9
Manganese Reduction	-11464.4	-16006.6	-4542.2
Sulfate Reduction	-4797.7	-4785.8	11.9
Methanogenesis	-1596.5	-1474.8	121.6

Dodecane	**ΔH_f (r)**	**ΔH_f (p)**	**ΔH^0**
Oxidation	-226.2	-8437.8	-8211.6
Denitrification	-3294.2	-10552.9	-7258.7
Manganese Reduction	-19466.2	-27179.4	-7713.2
Sulfate Reduction	-8636.3	-7882.7	753.6
Methanogenesis	-1798.3	-1774.0	24.2

APPENDIX J

MICROBIAL ENUMERATION DATA

Table J-1. Microbial Enumeration Data

MW	Test	Characteristic Number	Dilution (1^{st} value)	Min (95% CI)	Expected	Max (95%CI)
16	Total Heterotrophs	5-4-1	0.0001	6.0E+05	1.7E+06	3.9E+06
3		5-3-1	0.0001	3.0E+05	1.1E+06	2.4E+06
8		5-2-0	0.001	1.5E+04	4.9E+04	1.5E+05
16	Hydrocarbon Degraders	5-4-1	0.001	6.0E+04	1.7E+05	3.9E+05
3		5-3-0	0.01	2.3E+03	7.9E+03	2.2E+04
8		5-4-3	0.01	1.0E+04	2.8E+04	7.0E+04
16	Nitrate Reducers (nitrite)	5-5-5	0.00001	4.0E+06	1.6E+07	4.6E+07
3		5-5-5	0.00001	4.0E+06	1.6E+07	4.6E+07
8		5-5-5	0.00001	4.0E+06	1.6E+07	4.6E+07
16	Nitrate Reducers (N_2)	5-4-1	0.0001	6.0E+04	1.7E+05	3.9E+05
3		5-4-1	0.1	6.0E+01	1.7E+02	3.9E+02
8		5-2-3	0.01	3.0E+02	1.2E+03	2.4E+03
16	Iron Reducers	5-1-0	1	3.0E+01	1.3E+02	3.5E+02
3		4-2-0	1	7.0E+00	2.2E+01	4.8E+01
8		5-4-0	1	1.0E+01	3.3E+01	1.0E+02
16	Sulfate Reducers	0-0-0			0	
3		0-0-0			0	
8		0-0-0			0	

MPN Sample Calculation

Hydrocarbon Degraders
Well 8
Week 6
Temperature: 10°C

Score from positive and negative test tubes (Characteristic number): 5-2-0

The MPN tables in Alef and Nannipieri (1995) give the following values for this characteristic number:

Minimum 95% Confidence Interval = 15
Expected MPN = 49
Maximum 95% Confidence Interval = 149

The first positive score was found at a dilution of 0.001. The dilution correction is calculated as:

49 MPN/0.001 = 49,000 MPN

Thus, the MPN values are:

Minimum 95% Confidence Interval = 15,000
Expected MPN = 49,000
Maximum 95% Confidence Interval = 149,000

APPENDIX K

ANAEROBIC MICROBIAL ACTIVITY DATA

$^{14}CO_2$ MEASUREMENTS

$^{14}CO_2$ EXTRACTIONS

Table K-1. Percent Recovery of ^{14}C-dodecane as $^{14}CO_2$ in Anaerobic Microcosms Prior to Any Additions of Extra Free-Product and Radiolabel (*Incubation Temperature is 10°C unless otherwise specified)

	% Recovery at Day												
	0	**16**	**23**	**65**	**75**	**89**	**101**	**128**	**138**	**148**	**171**	**194**	**211**
Toluene A	6.30	0	6.18	0	1.13	0.22	0.48	0.83		0.00	1.12	0.87	0.90
Toluene B	1.81	0	2.65	0	1.24	0.73	0.49	0.87		0.28	3.36	1.06	1.88
Toluene C		0	6.45	2.90	0.84	4.28	0.86	1.11		0.21	0.68	0.73	2.62
Acetate A	6.78	26.77	26.79	43.54	71.46	71.62	74.11	22.58	24.57	54.44	53.83	29.33	51.75
Acetate B	8.57	6.07	10.93	32.60	52.62	42.83	45.38	34.49	52.43	65.02	26.02	37.65	32.56
Acetate C	5.12	18.80	25.11	18.80	46.25	49.25	55.11	22.42	30.47	50.94	50.38	57.10	56.91
Unamended 10° A	4.63	4.07	5.53	2.27	2.78	0.49	1.18	1.92		2.93	0.35	2.69	0.83
Unamended 10° B	4.76	0.72	4.02	0.69	3.03	4.12	0.04	1.10		0.28	4.17	5.56	2.37
Unamended 10° C	3.11	4.96	7.46	5.08	4.33	1.63	3.45	1.84		5.10	1.51	2.07	4.21
Unamended 23° A	2.36	1.46	0.40	7.10	5.60	1.60	1.16	10.77		0.35	3.32	3.35	3.49
Unamended 23°B	1.13	0.34		4.98	3.94	1.04	0.94	8.92			10.20	3.06	2.26
Unamended 23°C	2.15	4.54	0.35	2.14	5.63	5.03	6.15	10.45		1.37	4.73	3.77	4.55
Nutrient A	7.02	8.33	8.79	9.73	1.64	14.29	12.57	10.76		17.76	16.09	17.82	8.91
Nutrient B	0.66	12.64	13.81	1.23	0.43	14.81	2.55	8.91		17.17	10.24	14.66	4.63
Nutrient C	8.28	8.23	12.06	5.89	7.07	0.77	1.15	10.44		11.76	11.21	14.14	7.08
Nitrate A	3.13	2.02	0.85	0.76	1.66	0.72	1.33	1.11		2.50	7.18	3.62	1.61
Nitrate B	1.80	3.35	3.62	4.88	4.97	1.35	1.22	1.97		2.84	7.39	3.53	8.90
Nitrate C	5.34	2.73	3.34	3.70	3.36	1.06	0.96	5.33		2.36	5.66	4.78	6.39
Sulfate A	0.90	0.84	0.21	0	1.03	5.18	2.77	3.57		4.55	2.85	9.14	6.51
Sulfate B	2.64	5.37	5.78	0.15	0.88	9.98	10.19	1.70		2.61	6.21	7.80	8.42
Sulfate C	1.31	4.81	1.91	0	2.31	7.20	5.49	3.61		2.95	15.18	0.91	0.85
Negative Toluene	30.58	6.32	1.73	9.22	0.57	10.17	1.16			0.60		0.70	
Negative Acetate	10.23	17.73	3.76	14.04	3.63	12.97	3.23		5.35	3.62			
Negative Dodecane	0.62	1.67	0.14	1.62	0.07	1.97	0.10	0.07		0.05	0.04	0.07	0.10

Table K-1. Percent Recovery of ^{14}C-dodecane as $^{14}CO_2$ in Anaerobic Microcosms Prior to Any Additions of Extra Free-Product and Radiolabel

AVERAGES	0	16	23	65	75	89	101	128	138	148	171	194	211
Toluene	4.05	0	5.09	0.22	1.07	1.74	0.61	0.94		0.16	1.74	0.89	1.80
Acetate	6.82	17.21	20.94	31.64	56.78	54.56	58.20	26.50	35.82	56.80	43.41	41.36	47.07
Unamended 10°	4.17	3.25	5.67	2.68	3.38	2.08	1.55	1.62		2.77	2.01	3.44	2.47
Unamended 23°	1.88	2.11	0.38	4.74	5.05	2.56	2.75	10.05		0.86	6.09	3.39	3.43
Nutrient	5.32	9.73	11.55	5.62	3.05	9.96	5.43	10.04		15.56	12.51	15.54	6.87
Nitrate	3.42	2.70	2.60	3.11	3.33	1.04	1.17	2.80		2.56	6.74	3.97	5.63
Sulfate	1.62	3.67	2.63	0	1.41	7.46	6.15	2.96		3.37	8.08	5.95	5.26
Negative Toluene	30.58	6.32	1.73	9.22	0.57	10.17	1.16			0.60		0.70	
Negative Acetate	10.23	17.73	3.76	14.04	3.63	12.97	3.23		5.35	3.62			
Negative Dodecane	0.62	1.67	0.14	1.62	0.07	1.97	0.10	0.07		0.05	0.04	0.07	0.10

SD	0	16	23	65	75	89	101	128	138	148	171	194	211
Toluene	3.18	0.36	2.12	2.36	0.21	2.22	0.21	0.15		0.15	1.44	0.17	0.87
Acetate	1.73	10.44	8.71	12.40	13.11	15.11	14.61	6.92	14.68	7.33	15.16	14.25	12.83
Unamended 10°	0.92	2.24	1.72	2.22	0.84	1.86	1.74	0.45		2.42	1.96	1.86	1.69
Unamended 23	0.66	2.18	0.04	2.49	0.97	2.16	2.95	0.99		0.72	3.64	0.36	1.14
Nutrient	4.09	2.52	2.55	4.26	3.53	7.96	6.23	0.99		3.31	3.14	1.99	2.15
Nitrate	1.79	0.67	1.52	2.12	1.66	0.31	0.19	2.23		0.25	0.94	0.70	3.70
Sulfate	0.91	2.47	2.86	0.20	0.79	2.41	3.75	1.09		1.04	6.38	4.41	3.94

Table K-2. Complete Recovery (%) of 14C-Dodecane by Sequential Extraction With and Without Additions

		Toluene (DPM)	Dodecane (DPM)
1	Initial addition	68847	690912
2	2nd addition		143153
3	TOTAL		834065

Date	Microcosm Type	Incubation Time (from addition of radiolabel or f-p)	Type of additions		Recovery from flushing ($^{14}CO_2$)	Recovery from fluor (Volatilized ^{14}C during flushing)	Recovery from Extraction (^{14}C extracted from vial using DCM)	Recovery from Water (after extraction)	Recovery from stopper (chopped up and placed in fluor)	1 TOTAL (from initial radiolabel)	2 TOTAL (from 2nd addition of dodecane)	3 TOTAL (inital and extra dodecane)
8/14/2001	Toluene (68847 DPM)	19	none	A	1.2	38.7	13.6	2.0		55.4		
			f-p	B	1.4	33.6	19.2	1.2		55.4		
			f-p	C	1.1	31.1	32.8	1.5		66.6		
9/25/2001	Toluene (68847 DPM)	62	f-p	A	1.4	60.5	30.2	0.8	2.3	95.1		
				B	0.3	6.2	43.4	0.7	7.8	58.4		
				C		36.0	17.7	0.0	6.6	60.3		
5/29/2001	Unamended 10C (690912 DPM)	210	none	A	0.4	0.0	0.5	1.2	0.2	2.3		
				B	1.0	0.1	0.4	1.1	0.1	2.7		
				C	1.8	0.1	0.3	1.5	0.1	3.8		
8/14/2001	Unamended 10C (690912 DPM)	19	f-p	A	1.7	0.1	2.4	1.2		5.4		
				B	0.0	0.1	1.0	0.6		1.8		
8/14/2001	Unamended 10C (143153 DPM)	19	f-p and C14	A	13.4	23.2	54.0	5.1			95.7	16.4
				B	5.2	20.8	61.4	2.7			90.1	15.5
9/25/2001	Unamended 10C (143153 DPM)	62	f-p and C14	A	9.2	35.6	78.5	3.7	1.6		128.6	22.1
				B	18.4	32.7	87.2	3.3	3.7		145.2	24.9
				C	9.0	36.3	56.8	6.3	1.3		109.8	18.8
9/27/2001	Unamended 10C (143153 DPM)	28	f-pand C14 (inverted)	A	12.7	14.6	39.5	4.1	3.6		74.4	12.8
				B	4.8	14.5	66.4	2.2	1.2		98.1	15.3
				C	14.6	13.7	59.2	5.8	2.0		95.3	16.4
5/29/2001	Unamended 23C (690912 DPM)	210	none	A	1.5	0.0	0.3	1.8	1.1	4.8		
				B	1.0	0.1	0.5	1.3	2.6	5.5		
				C	2.0	0.1	0.2	1.7	0.3	4.3		
8/14/2001	Unamended 23C (690912 DPM)	19	f-p	A	4.4	1.0	0.9	2.1		8.4		
				B	0.0	1.7	1.0	0.5		3.1		
8/14/2001	Unamended 23C (143153 DPM)	19	f-p and C14	A	3.9	19.7	79.1	2.2			104.9	18.0
				B	21.4	4.8	68.2	2.7			97.1	16.7
9/24/2001	Unamended 23C (143153 DPM)	61	f-p and C14	A	2.1	1.1	4.0	0.0	36.3		41.4	7.6
				B	5.8	25.7	54.6	0.1	7.6		87.9	16.5
				C	2.9	11.6	14.7	0.1	32.6		59.0	11.1
9/27/2001	Unamended 23C (143153 DPM)	28	f-pand C14 (inverted)	A	11.0	16.6	46.4	4.6	5.9		84.5	14.5
				B	9.4	21.9	64.7	4.7	10.1		110.8	19.0
				C	5.7	19.3	52.9	3.2	4.6		85.8	14.7

Table K-2. Complete Recovery (%) of 14C-Dodecane by Sequential Extraction With and Without Additions

Date	Microcosm Type	Incubation Time (from addition of radiolabel or f-p)	Type of additions		Recovery from flushing ($^{14}CO_2$)	Recovery from fluor (Volatilized ^{14}C during flushing)	Recovery from Extraction (^{14}C extracted from vial using DCM)	Recovery from Water (after extraction)	Recovery from stopper (chopped up and placed in fluor)	TOTAL (from initial radiolabel)	TOTAL (from 2nd addition of dodecane)	TOTAL (inital and extra dodecane)
5/29/2001	Nutrient (690912 DPM)	210	none	A	0.7	0.0	0.4	0.6	0.3	2.5		
				B	3.8	0.1	0.3	1.4	0.3	5.9		
				C	2.7	0.1	0.3	1.1	1.6	5.9		
8/8/2001	Nutrient (690912 DPM)	13	f-p	A	1.3	1.2	0.6	0.2		3.4		
				B	0.1	0.9	2.8	0.6		4.3		
08/08/01	Nutrient (143153 DPM)	13.0	f-p and C14	A	0.0	15.5	82.5	2.2			100.2	11.1
				B	0.1	20.1	94.7	1.6			116.5	13.3
9/24/2001	Nutrient (143153 DPM)	61	f-p and C14	A	30.6	31.1	60.2	2.1	3.2		127.2	21.8
				B	19.7	28.2	61.4	1.3	1.9		112.5	19.3
				C	3.0	32.7	104.5	0.6	13.3		154.0	26.4
9/28/2001	Nutrient (143153 DPM)	29	f-pand C14 (inverted)	A	6.0	4.6	43.6	1.2	0.9		56.3	9.7
				B	6.2	7.7	34.3	1.4	1.9		51.5	8.8
				C	17.5	13.9	46.8	1.7	3.5		83.3	14.3
5/29/2001	Nitrate (690912 DPM)	210	none	A	0.7	0.0	0.4	0.1	0.2	1.5		
				B	3.8	0.1	1.2	0.2	[illegible]	5.8		
				C	2.7	0.1	0.8	0.2	0.9	4.8		
8/8/2001	Nitrate (690912 DPM)	13	f-p	A	6.3	1.3	1.4	0.5		9.5		
				B	0.0	0.4	0.5	0.5		1.5		
8/8/2001	Nitrate (143153 DPM)	13	f-p and C14	A	12.2	15.2	54.1	0.9			82.5	14.2
				B	10.7	15.0	85.4	12.6			123.7	21.2
9/25/2001	Nitrate (143153 DPM)	62	f-p and C14	A	13.7	31.9	119.5	0.8	4.3		170.2	29.2
				B	53.8	24.6	90.9	4.2	12.0		185.6	31.9
				C	10.5	40.8	95.6	1.8	1.7		150.3	25.8
9/28/2001	Nitrate (143153 DPM)	29	f-pand C14 (inverted)	A	23.0	12.4	3.9	2.4	1.8		105.9	18.2
				B	23.9	16.1	2.3	2.3	0.5		104.4	17.9
				C	27.9	14.2	3.8	2.1	1.3		105.3	18.1
5/29/2001	Sulfate (690912 DPM)	210	none	A	2.8	0.2	0.4	1.4	0.1	4.9		
				B	3.6	0.0	0.3	2.0	0.1	6.2		
				C	0.4	0.0	0.2	0.6	0.1	1.4		
8/14/2001	Sulfate (690912 DPM)	19	f-p	A	8.9	1.1	2.4	2.4		14.8		
				B	0.0	1.1	1.0	0.2		2.2		
8/14/2001	Sulfate (143153 DPM)	19	f-p and C14	A	16.3	26.1	76.4	9.3			128.1	22.0
				B	2.2	23.7	75.1	2.5			103.5	17.8
9/25/2001	Sulfate (143153 DPM)	62	f-p and C14	A	2.3	44.1	96.9	5.9	2.5		151.7	26.0
				B	12.2	34.2	66.2	5.0	4.7		122.4	21.0
				C	10.8	37.4	48.7	3.6	6.4		106.9	18.4
9/27/2001	Sulfate (143153 DPM)	28	f-pand C14 (inverted)	A	19.6	13.1	66.9	7.0	4.6		111.1	19.1
				B	26.1	10.1	66.9	11.3	5.2		92.6	15.9
				C	4.2	5.0	64.4	2.6	3.5		79.7	12.1

Table K-2. Complete Recovery (%) of 14C-Dodecane by Sequential Extraction With and Without Additions

Date	Microcosm Type	Incubation Time (from addition of radiolabel or f-p)	Type of additions		Recovery from flushing ($^{14}CO_2$)	Recovery from fluor (Volatilized ^{14}C during flushing)	Recovery from Extraction (^{14}C extracted from vial using DCM)	Recovery from Water (after extraction)	Recovery from stopper (chopped up and placed in fluor)	TOTAL (from initial radiolabel)	TOTAL (from 2nd addition of dodecane)	TOTAL (inital and extra dodecane)
5/28/2001	Negative Dodecane (690911.6 CPM)	209	none	A	0.0	0.3	0.3	1.0	97.2	98.7		
8/8/2001	Negative Dodecane (143153 DPM)	13	f-p and C14	A	1.1	12.3	78.3	0.5			93.6	16.2
9/24/2001	Negative Dodec. (143153 DPM)	61	f-p and C14	A	0.5	32.0	126.3	0.6	4.7		163.6	28.2
9/28/2001	Negative Dodec. (143153 DPM)	29	f-pand C14 (inverted)	A	1.2	13.1	59.8	1.3	1.3		76.7	13.2
9/28/2001	Sterile 10 C (143153 DPM)	29	fp and C14 right side up	A	1.5	19.3	31.8	1.0	2.3		55.8	9.6
				B	0.2	23.8	36.3	0.5	1.8		62.5	10.7
9/28/2001	Sterile 35 C (143153 DPM)	29	fp and C14 right side up	A	0.1	24.0	37.1	1.4	1.9		64.6	11.1
				B	0.1	21.7	40.8	1.2	1.9		65.7	11.3

Table K-3. Origin of Radiolabelled Dodecane After the Addition of Extra Radiolabelled Dodecane. (See p.290 for sample calculations)

	Dodecane
Initial (1)	690911.6
2nd addition (2)	143153.3
TOTAL	834064.9

Microcosm Type	Incubation Time (from addition of radiolabel or f-p)	Type of additions		Counts (dpm) after 2nd addition	% Total Recovery from 2nd addition of dodecane (2)	Counts (dpm) Remaining	% of ^{14}C-Dodecane from initial docdecane (1)
Unamended 10C (143153 DPM)	19	f-p and C14	A	137035.0	95.7	0.0	
			B	129006.0	90.1	0.0	
Unamended 10C (143153 DPM)	62	f-p and C14	A	18074.5	128.6[a]	**12290.5[b]**	**1.8[c]**
			B	207838.5	145.2	**36054.5**	**5.2**
			C	157158.0	109.8	0.0	
Unamended 10C (143153 DPM)	28	f-pand C14 (inverted)	A	106540.5	74.4	0.0	
			B	140491.0	98.1	0.0	
			C	136483.0	95.3	0.0	
Unamended 23C (143153 DPM)	19	f-p and C14	A	150179.0	104.9	0.0	
			B	139015.0	97.1	0.0	
Unamended 23C (143153 DPM)	61	f-p and C14	A	59252.0	41.4	0.0	
			B	125900.0	87.9	0.0	
			C	84400.0	59.0	0.0	
Unamended 23C (143153 DPM)	28	f-pand C14 (inverted)	A	120948.0	84.5	0.0	
			B	158661.0	110.8	0.0	
			C	122835.5	85.8	0.0	
Nutrient (143153 DPM)	13	f-p and C14	A	143493.0	100.2	0.0	
			B	166797.0	116.5	0.0	
Nutrient (143153 DPM)	61	f-p and C14	A	182048.0	127.2	**10264.0**	**1.5**
			B	161011.0	112.5	0.0	
			C	220462.0	154.0	**48678.0**	**7.0**
Nutrient (143153 DPM)	29	f-pand C14 (inverted)	A	80597.0	56.3	0.0	
			B	73713.0	51.5	0.0	
			C	119237.0	83.3	0.0	
Nitrate (143153 DPM)	13	f-p and C14	A	118063.0	82.5	0.0	
			B	177047.0	123.7	**5263.0**	**0.8**
Nitrate (143153 DPM)	62	f-p and C14	A	243675.5	170.2	**71891.5**	**10.4**
			B	265663.0	185.6	**93879.0**	**13.6**
			C	215154.0	150.3	**43370.0**	**6.3**
Nitrate (143153 DPM)	29	f-pand C14 (inverted)	A	151624.0	105.9	0.0	
			B	149412.0	104.4	0.0	
			C	150781.0	105.3	0.0	

Table K-3. Origin of Radiolabelled Dodecane After the Addition of Extra Radiolabelled Dodecane. (See p.290 for sample calculations)

Microcosm Type	Incubation Time (from addition of radiolabel or f-p)	Type of additions		Counts (dpm) after 2nd addition	% Total Recovery from 2nd addition of dodecane (2)	Counts (dpm) Remaining	% of ^{14}C-Dodecane from initial docdecane (1)
Sulfate (143153 DPM)	19	f-p and C14	A	183388.0	128.1	**11604.0**	**1.7**
			B	148095.5	103.5	0.0	
Sulfate (143153 DPM)	62	f-p and C14	A	217167.5	151.7	**45383.5**	**6.6**
			B	175203.0	122.4	**3419.0**	**0.5**
			C	153064.0	106.9	0.0	
Sulfate (143153 DPM)	28	f-pand C14 (inverted)	A	159033.5	111.1	0.0	
			B	132602.0	92.6	0.0	
			C	114152.0	79.7	0.0	
Negative Dodecane (143153 DPM)	13	f-p and C14	A	133925.9	93.6	0.0	
						0.0	
Negative Dodecane (143153 DPM)	61	f-p and C14	A	234256.0	163.6	**62472.0**	**9.0**
						0.0	
Negative Dodecane (143153 DPM)	29	f-pand C14 (inverted)	A	109756.5	76.7	0.0	
Sterile 10 C (143153 DPM)	29	fp and C14 right side up	A	79897.0	55.8	0.0	
			B	89529.0	62.5	0.0	
Sterile 35 C (143153 DPM)	29	fp and C14 right side up	A	92445.0	64.6	0.0	
			B	94105.5	65.7	0.0	

Sample calculation to determine distribution and origin of radiolabelled dodecane

Recoveries after the addition of extra radiolabelled dodecane sometimes exceeded 100%. This extra radiolabel can be attributed to the initial addition of ^{14}C-Dodecane in methanol. Calculations were carried out to determine how much radiolabel was from each addition.

In Quality Control (QC) in commercial labs, acceptable percentage recovery from standards often falls in the range of 80-120%. Assuming that up to 120% of ^{14}C-dodecane dissolved in the recently added free-product could be recovered, anything above this value could be attributed to the original ^{14}C-dodecane addition (dissolved in methanol).

If it were assumed that all of addition 2 of radiolabelled dodecane (143153 dpm) was recovered up to 120 %, then the maximum dpm detected would be:

143153 dpm x 120% = 171783.96 dpm

The origin of the radiolabelled dodecane was calculated as follows (refer to Table K-3 for values)

a) % total recovery based on 2nd addition of dodecane only =
184075/143153 dpm = 128.6%

b) Counts (dpm) remaining (assuming 120% recovery from 2nd addition) =
184075 dpm - 120%(143153dpm) = 12291 dpm

c) % of ^{14}C-dodecane from the initial addition = 12291/690912 dpm = 1.8%

APPENDIX L

ANAEROBIC MICROBIAL ACTIVITY DATA FROM 1-L MICROCOSMS

Original Amendments
Nitrate = 303 mg/L
Sulfate = 426 mg/L

All samples were amended with 45 mg/L of free-product on November 16th, 2000

Table L-1. Concentrations of Nitrate, Sulfate, TEH and TPH in 1-L Microcosms

NITRATE (mg/L)	Amended with 45 mg/L of free-product on November 16th, 2000					
	14/11/00	14/11/00	09/07/01	09/07/01	06/09/01	06/09/01
SAMPLE	0A	0B	1A	1B	2A	2B
Negative	0.134	0.019	0.013	destroyed	0.015	<0.003
Unamended 10C	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
Unamended 20C	0.015	<0.003	<0.003	<0.003	<0.003	<0.003
Nutrient	32.1	26.9	16.2	14.8	3.48	0.137
Nitrate	45	39.3	23.3	25.7	24.4	17.5
Sulfate	<0.003	<0.003	<0.003	<0.003	<0.003	0.005

SULPHATE (mg/L)	14/11/00	14/11/00	09/07/01	09/07/01	06/09/01	06/09/01
SAMPLE	0A	0B	1A	1B	2A	2B
Negative	<1	<1	0.2	destroyed	0.5	0.8
Unamended 10C	<1	<1	<0.1	0.9	0.2	<0.1
Unamended 20C	<1	<1	<0.1	1.2	0.1	0.1
Nutrient	<1	<1	0.5	0.6	0.5	0.5
Nitrate	1.7	<1	0.7	0.3	1.6	2.2
Sulfate	330	310	93.3	195	260	263

TEH (mg/L)	14/11/00	14/11/00	09/07/01	09/07/01	06/09/01	06/09/01
SAMPLE	0A	0B	1A	1B	2A	2B
Negative	<2	<2	44.53	destroyed	40.8	49.9
Unamended 10C	<2	<2	62.3	51.7	44.4	38.5
Unamended 20C	<2	<2	40.8	44.1	47.6	45.7
Nutrient	<2	<2	34.2	24.7	27	27.2
Nitrate	<2	<2	33.3	40	30.9	32.5
Sulfate	<2	<2	49.4	50.8	39.5	41.3

TPH (mg/L)	14/11/00	14/11/00	09/07/01	09/07/01	06/09/01	06/09/01
SAMPLE	0A	0B	1A	1B	2A	2B
Negative	<0.1	<0.1	0.2	destroyed	1	0.3
Unamended 10C	0.2	0.2	<0.1	0.4	0.7	0.8
Unamended 20C	0.2	0.3	<0.1	<0.1	0.4	1
Nutrient	0.1	0.4	<0.1	<0.1	0.3	0.3
Nitrate	0.2	0.1	<0.1	<0.1	<0.1	<0.1
Sulfate	0.2	0.3	0.1	<0.1	<0.1	<0.1

APPENDIX M

METABOLITE DATA FROM DR. LISA GIEG

Samples: Alberta samples from site 4362: comparison of Fall 2000 and May 2001 samples. All samples are from well 3.
Sampling date: Fall 2000 and May 2001
Extraction and analysis: February 2-4 and June 11-12, 2001
Details: Both samples were sent from Katharine Cross (U of A Engineering); Fall '00 samples had been stored at 4°C, May '01 samples had just been sampled

"X" indicates that metabolite was detected

Possible metabolites: monoaromatics

Anaerobic:	**characteristic fragment ion**	**Fall '00 A**	**May '01 A**	**May '01 B Duplicate**
Benzylsuccinic acid	337			
E-phenylitaconic acid	335			
Ethylbenzylsuccinic acid	159 and 351			
2-methylbenzylsuccinic acid	159 and 351			
3-methylbenzylsuccinic acid	159 and 351	X		
4-methylbenzylsuccinic acid	159 and 351			
Other	159			
Cyclohexane-COOH	185			
Cyclohexene-COOH	183			
2-OH-cyclohexane-COOH	273			

Aerobic or anaerobic:	**characteristic fragment ion**	**Fall '00 A**	**May '01 A**	**May '01 B Duplicate**
Benzoate	179	X	X	X
Benzylalcohol	165			
Benzaldehyde	106			
Methylbenzaldehydes	119			
Acetophenone	105			
1-phenylethanol	179			
cinnamic acid	205			
Hydrocinnamic acid	207			
Phenylacetic acid	193			
Tolylacetic acid	207			
Phenol	151			
o-cresol	165			
m-cresol	165	X		
p-cresol	165			
o-toluate	193	X	X	X
m-toluate	193	X	X	X
p-toluate	193	X	X	X

		Fall '00	May '01	
Aerobic or anaerobic:	**characteristic fragment ion**	**A**	**A**	**B Duplicate**
o-phthalate	295			
m-phthalate	295	**X**		
p-phthalate	295			
Dimethylphenols	179	**X**		
Carboxybenzaldehyes	207	**X**		**X**
Aerobic:				
Catechol	254			
Methylcatechol	268			

Possible metabolites: alkanes

		Fall '00	May '01	
Aerobic or anaerobic:	**characteristic fragment ion**	**A**	**A**	**B Duplicate**
C1: methylsuccinic acid	261			
C2: ethylsuccinic acid	262 and 275			
C3: propylsuccinic acid	262 and 289			
C4: butylsuccinic acid	262 and 303			
C5: pentylsuccinic acid	262 and 317			
C5 w/ unsat	262 and 315			
C6: hexylsuccinic acid	262 and 331			
C6 w/ unsat	262 and 329			
C7: heptylsuccinic acid	262 and 345			
C7 w/ unsat	262 and 343			
C8: octylsuccinic acid	262 and 359			
C8 w/ unsat	262 and 357			
C9: nonylsuccinic acid	262 and 373			
C9 w/ unsat	262 and 371			
C10: decylsuccinic acid	262 and 387			
C11: undecylsuccinic acid	262 and 401			
C12: dodecylsuccinic acid	262 and 415			
Aerobic or anaerobic:				
Monoalkanoic acids				
Hexanoic acid	117 and 173			
Octanoic acid	117 and 201			
Decanoic acid	117 and 229			
Dodecanoic acid	117 and 257			
Tetradecanoic acid	117 and 285			
Hexadecanoic acid	117 and 313			
Heptadecanoic acid	117 and 327			
Octadecanoic acid	117 and 341			

	Characteristic fragment ion	Fall '00 samples A	May '01 samples A	 B Duplicate
Dialkanoic acids				
Succinic acid (C4)	247			
Glutaric acid (C5)	261			
Adipic acid (C6)	275			
Pimelic acid (C7)	289			
Octanedioic acid (C8)	303			
Azeleic acid (C9)	317			
Sebacic acid (C10)	331			
Undecanedioic acid (C11)	345			
Dodecanedioic acid (C12)	359			
Possible metabolites: naphthalenes				
1-naphthoic acid	229			
2-naphthoic acid	229	X		
Methylnaphthoic acid	243	X	X	X
Dimethylnaphthoic acid	257	X		
1,2,3,4-tetrahydronaphthoic acid	130	X	X	X
5,6,7,8-tetrahydronaphthoic acid	233	X	X	X
5,6,7,8-tetrahydromethylnaphthoic acid	247	X		
Decahydronaphthoic acid	239			
Methyldecahydronaphthoic acid	253			
Aerobic:				
salicylic acid	267			

APPENDIX N

AEROBIC MICROBIAL ACTIVITY DATA $^{14}CO_2$ MEASUREMENT

Table N-1. Percent Recovery of ^{14}C-dodecane as $^{14}CO_2$ (Aerobic Biodegradation)

Day	1	2	3	4	7	11	15	20	25	28
Unamended 10 A	0.01	0.08	0.13	0.10	0.05	0.32	0.12	0.19	0.20	0.32
Unamended 10 B	0.04	0.06	0.08	0.16	0.07	0.14	0.22	0.17	0.16	0.15
Unamended 10 C	0.06	0.05	0.06	0.04	0.04	0.10	0.13	0.16	0.15	0.16
Nutrient 10 A	0.00	0.04	0.03	0.04	0.04	0.26	0.39	0.52	0.75	0.97
Nutrient 10 B	0.06	0.01	0.05	0.09	0.07	0.36	0.75	1.42	2.08	1.79
Nutrient 10 C	0.01	0.00	0.09	0.11	0.12	0.35	0.55	1.16	1.76	2.16
Unamended 28 A	0.01	0.04	0.05	0.09	0.06	0.09	0.07	0.12	0.12	0.16
Unamended 28 B	0.02	0.05	0.02	0.12	0.21	0.32	0.37	0.46	0.52	0.58
Unamended 28 C	0.00	0.04	0.06	0.08	0.12	0.28	0.19	0.24	0.30	0.33
Nutrient 28 A	0.07	0.14	0.12	0.47	1.06	3.91	6.02	6.74	11.47	12.80
Nutrient 28 B	0.06	0.10	0.21	0.51	1.18	4.96	7.50	11.72	11.86	15.84
Nutrient 28 C	0.06	0.11	0.09	0.27	0.60	3.16	4.37	6.58	9.86	11.51
Positive A	0.36	0.97	1.77	4.08	8.55	6.32	13.82	16.10	18.41	17.10
Positive B	0.13	0.77	2.00	4.17	4.85	6.57	14.35	9.59	16.77	22.45
Negative 10	0.07	0.04	0.04	0.06	0.04	0.18	0.05	0.13	0.09	0.11
Negative 28	0.02	0.01	0.04	0.12	0.08	0.06	0.05	0.10	0.09	0.16

Day	32	36	41	46	53	61	68	75	81	90
Unamended 10 A	0.17	0.29	0.36	0.35	0.49	4.17	2.16	1.07	1.18	1.21
Unamended 10 B	0.19	0.20	0.28	0.25	0.34	3.80	1.53	2.10	0.94	0.90
Unamended 10 C	0.17	0.32	0.28	0.34	0.44	1.56	1.54	3.28	0.89	0.93
Nutrient 10 A	1.51	1.78	3.02	3.53	5.03	7.62	7.99	9.00	9.20	10.83
Nutrient 10 B	3.34	4.32	5.78	7.16	10.99	12.56	14.54	15.71	17.65	19.00
Nutrient 10 C	3.29	4.96	6.66	7.83	9.90	13.68	13.94	16.24	16.73	18.11

Table N-1 Continued

Day	32	36	41	46	53	61	68	75	81	90
Unamended 28 A	0.15	0.30	0.35	0.41	0.51	4.17	2.95	2.93	1.60	1.74
Unamended 28 B	0.47	0.64	0.79	0.77	0.89	1.34	3.47	3.08	1.59	1.52
Unamended 28 C	0.30	0.45	0.56	0.59	0.74	3.20	5.64	2.58	1.71	1.93
Nutrient 28 A	13.64	16.63	18.60	21.54	24.49	26.56	30.37	31.51	30.90	29.63
Nutrient 28 B	16.04	11.49	22.27	25.28	26.14	31.39	32.53	34.58	35.24	35.87
Nutrient 28 C	10.00	14.91	16.26	17.58	19.66	21.83	22.15	24.37	26.04	23.57
Positive A	17.73	22.31	25.10	25.76	29.43	29.56	29.25	31.53	31.39	34.40
Positive B	23.84	22.51	24.94	27.20	30.41	30.61	31.31	33.50	33.86	35.64
Negative 10	0.08	0.09	0.14	0.11	0.13	0.33	0.15	0.33	0.18	0.21
Negative 28	0.08	0.08	0.19	0.09	0.09	1.27	1.58	0.59	0.42	0.41

Day	97	105	111	119	123	130	141	159	168	187
Unamended 10 A	1.24	1.42	1.62	1.29	1.23	1.55	1.67	2.08	2.37	2.45
Unamended 10 B	1.03	0.94	1.00	1.06	1.02	1.06	1.18	1.32	1.37	1.36
Unamended 10 C	0.97	0.94	1.02	1.02	1.04	1.08	1.15	1.20	1.24	1.18
Nutrient 10 A	12.42	13.71	15.30	13.89	14.51	18.42	20.96	25.96	25.75	30.22
Nutrient 10 B	22.15	23.33	24.09	22.99	23.41	26.80	26.85	30.42	30.82	33.56
Nutrient 10 C	20.00	20.02	21.33	20.14	20.79	23.75	23.72	27.04	28.04	31.27
Unamended 28 A	1.91	2.00	2.14	2.24	2.17	2.58	2.29	2.64	2.79	2.73
Unamended 28 B	1.64	1.65	1.66	1.62	1.65	1.77	1.83	1.99	1.98	2.02
Unamended 28 C	2.29	2.28	2.56	2.37	2.48	2.91	2.92	3.05	2.64	3.88
Nutrient 28 A	31.56	30.56	32.79	32.48	34.39	36.91	35.66	36.46	37.00	37.58
Nutrient 28 B	36.96	33.81	38.76	36.91	37.25	40.75	38.45	40.92	41.12	42.29
Nutrient 28 C	26.80	27.22	29.32	28.39	28.87	31.27	31.13	31.99	33.03	34.91
Positive A	32.86	32.94	33.10	34.88	34.10	36.93	37.15	39.51	39.63	40.33

Table N-1. Continued

Day	97	105	111	119	123	130	141	159	168	187
Positive B	36.09	37.33	37.25	36.04	37.06	40.45	40.25	42.19	42.99	43.20
Negative 10	0.20	0.16	0.18	0.17	0.14	0.14	0.14	0.15	0.15	0.16
Negative 28	0.46	0.38	0.47	0.60	0.53	0.55	0.74	0.40	0.40	0.41

Average	Day 1	2	3	4	7	11	15	20	25	28
Unamended 10	0.03	0.06	0.09	0.10	0.05	0.19	0.16	0.18	0.17	0.21
Unamended 28	0.01	0.04	0.04	0.09	0.13	0.23	0.21	0.27	0.31	0.36
Nutrient 10	0.02	0.02	0.06	0.08	0.08	0.32	0.56	1.04	1.53	1.64
Nutrient 28	0.06	0.12	0.14	0.42	0.95	4.01	5.96	8.35	11.06	13.38
Positive	0.25	0.87	1.88	4.12	6.70	6.45	14.09	12.85	17.59	19.78
Negative 10	0.07	0.04	0.04	0.06	0.04	0.18	0.05	0.13	0.09	0.11
Negative 28	0.02	0.01	0.04	0.12	0.08	0.06	0.05	0.10	0.09	0.16

Average	Day 32	36	41	46	53	61	68	75	81	90
Unamended 10	0.18	0.27	0.31	0.31	0.42	3.17	1.74	2.15	1.00	1.01
Unamended 28	0.30	0.47	0.57	0.59	0.71	2.90	4.02	2.86	1.63	1.73
Nutrient 10	2.71	3.68	5.15	6.17	8.64	11.29	12.16	13.65	14.53	15.98
Nutrient 28	13.23	14.34	19.04	21.47	23.43	26.59	28.35	30.15	30.73	29.69
Positive	20.78	22.41	25.02	26.48	29.92	30.08	30.28	32.52	32.62	35.02
Negative 10	0.08	0.09	0.14	0.11	0.13	0.33	0.15	0.33	0.18	0.21
Negative 28	0.08	0.08	0.19	0.09	0.09	1.27	1.58	0.59	0.42	0.41

Average	Day 97	105	111	119	123	130	141	159	168	187
Unamended 10	1.08	1.10	1.21	1.12	1.10	1.23	1.34	1.53	1.66	1.66
Unamended 28	1.94	1.98	2.12	2.08	2.10	2.42	2.34	2.56	2.47	2.88

Table N-1. Continued

Average	Day 97	105	111	119	123	130	141	159	168	187
Nutrient 10	18.19	19.02	20.24	19.01	19.57	22.99	23.84	27.81	28.20	31.68
Nutrient 28	31.77	30.53	33.62	32.59	33.51	36.31	35.08	36.46	37.05	38.26
Positive	34.47	35.14	35.18	35.46	35.58	38.69	38.70	40.85	41.31	41.77
Negative 10	0.20	0.16	0.18	0.17	0.14	0.14	0.14	0.15	0.15	0.16
Negative 28	0.46	0.38	0.47	0.60	0.53	0.55	0.74	0.40	0.40	0.41

SD	Day 1	2	3	4	7	11	15	20	25	28
Unamended 10	0.03	0.01	0.03	0.06	0.01	0.12	0.06	0.02	0.03	0.10
Unamended 28	0.01	0.01	0.02	0.02	0.08	0.12	0.15	0.17	0.20	0.21
Nutrient 10	0.03	0.02	0.03	0.04	0.04	0.06	0.18	0.46	0.69	0.61
Nutrient 28	0.01	0.02	0.07	0.13	0.31	0.90	1.57	2.92	1.06	2.23
Positive	0.16	0.15	0.17	0.07	2.62	0.18	0.37	4.61	1.16	3.78

SD	Day 32	36	41	46	53	61	68	75	81	90
Unamended 10	0.01	0.06	0.05	0.05	0.079	1.41	0.36	1.11	0.15	0.17
Unamended 28	0.16	0.17	0.22	0.18	0.19	1.44	1.43	0.26	0.07	0.20
Nutrient 10	1.04	1.68	1.90	2.31	3.17	3.22	3.62	4.03	4.63	4.48
Nutrient 28	1.70	3.63	2.59	2.64	1.17	3.41	1.52	2.17	4.60	6.15
Positive	4.32	0.15	0.11	1.02	0.69	0.74	1.46	1.39	1.74	0.88

SD	Day 97	105	111	119	123	130	141	159	168	187
Unamended 10	0.14	0.28	0.35	0.14	0.12	0.28	0.29	0.48	0.62	0.69
Unamended 28	0.33	0.32	0.45	0.40	0.42	0.59	0.55	0.53	0.44	0.94
Nutrient 10	5.11	4.89	4.49	4.65	4.58	4.24	2.95	2.33	2.54	1.71
Nutrient 28	5.08	3.30	4.77	4.26	4.26	4.77	3.70	4.47	4.05	3.74
Positive	2.28	3.11	2.94	0.82	2.09	2.49	2.20	1.90	2.37	2.02

Sample Calculation to Convert dpm to % $^{14}CO_2$

Step	1	2	3	4	5	6	7
Day	Volume of KOH remaining (mL)	Counts (dpm) in blank	Counts (dpm) in sample	Sample –Blank (dpm)	Total Counts/ Volume of KOH	Cumulative Counts	(Cumulative Counts/ Initial dpm)*100 = % $^{14}CO_2$
1	10	38	47	9	180	180	0.071346
2	9.5	37	55	18	342	351	0.139124
3	9	36	52	16	288	315	0.124855
4	8.5	32	99	67	1139	1182	0.468504
7	8	39	200	161	2576	2686	1.064637
11	7.5	33	673	640	9600	9871	3.912522

Step 1: Record volume of KOH remaining in side arm test tube of biometer

Step 2: Record number of counts (dpm) in blank scintillation vial

Step 3: Record number of counts (dpm) found in scintillation vial containing 0.5 mL of sample

Step 4: Subtract sample counts from blank (dpm)

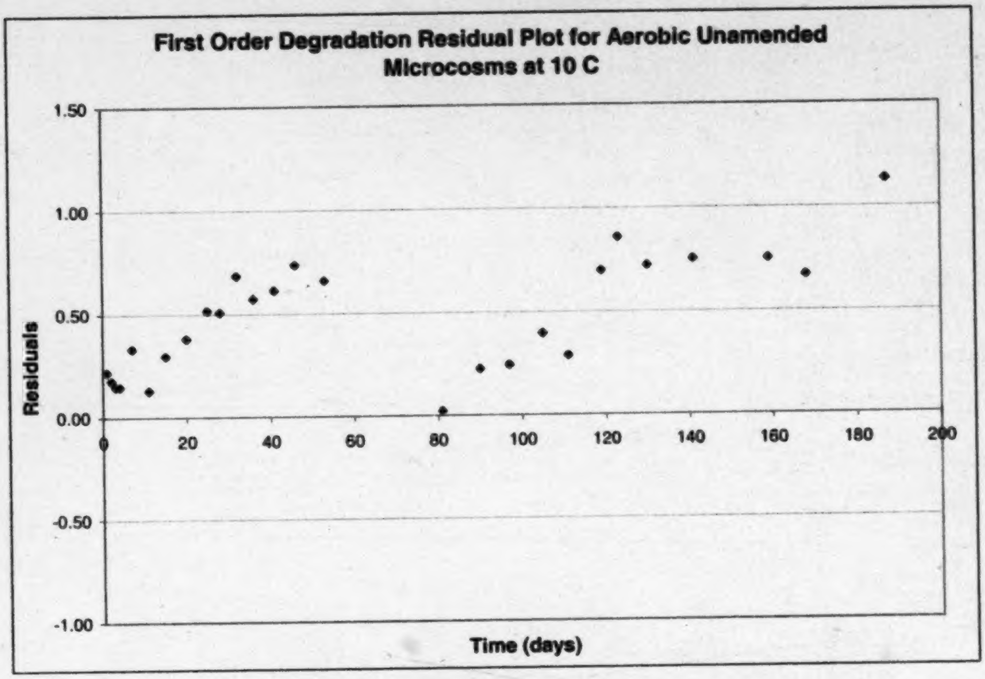
Step 5: Divide the total counts found in step 4 by the volume of KOH in the test tube

Step 6: Add the value found in Step 5 to all previous values found in step 4 (*ie*. for day 11 cumulative counts =9871+161+67+16+18+9)

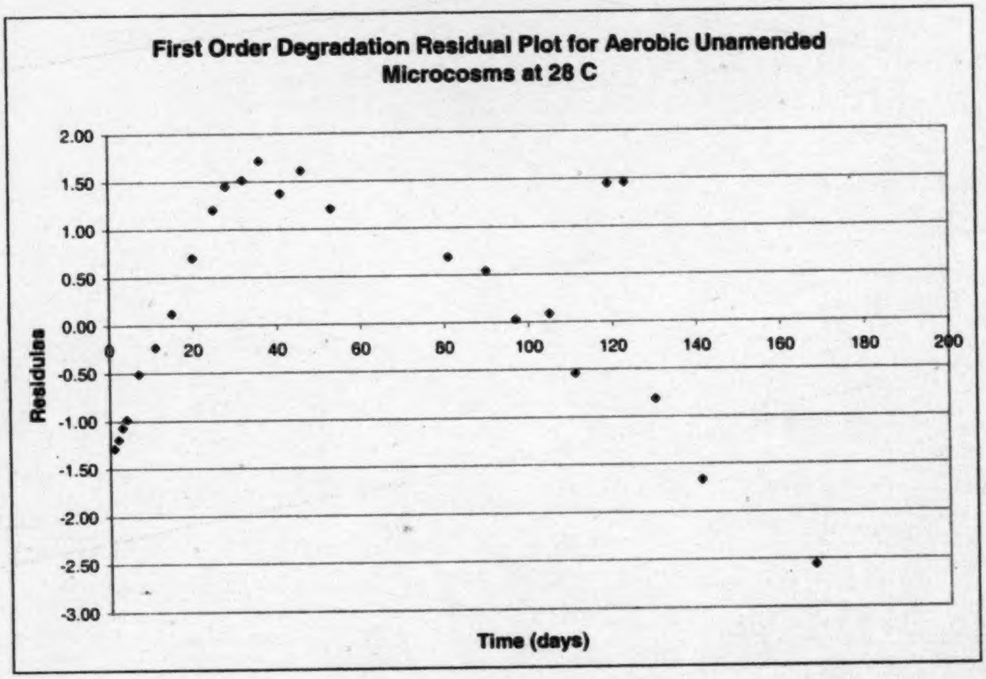
Step 7: Divide the cumulative counts by the initial number of counts (dpm) added to the biometer (*ie*. 252293 dpm), then multiply by 100 to find % $^{14}CO_2$

APPENDIX O

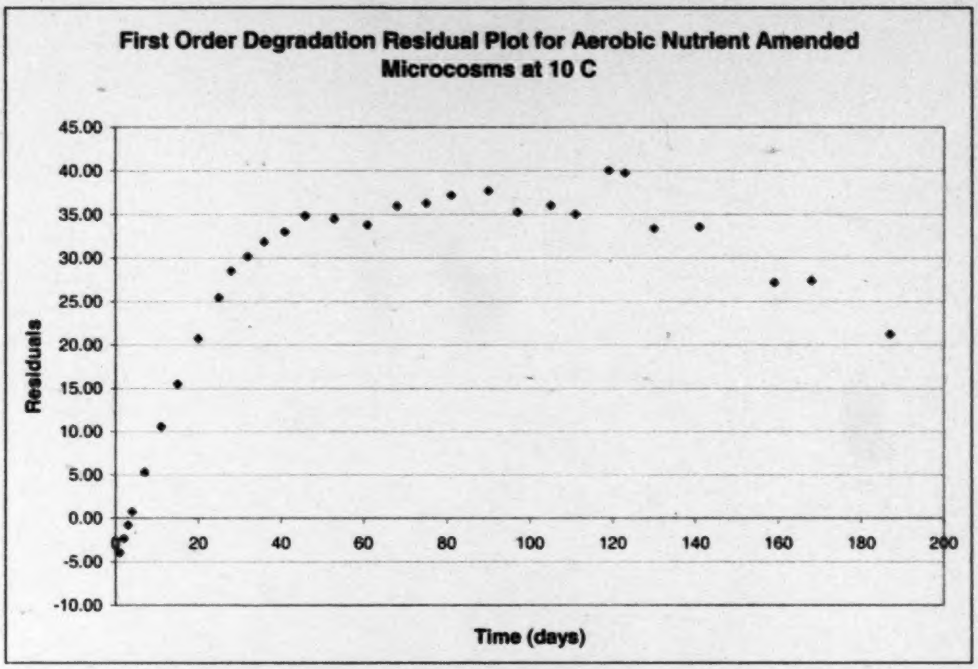
RESIDUAL PLOTS FOR THE FIRST ORDER BIODEGRADATION MODEL

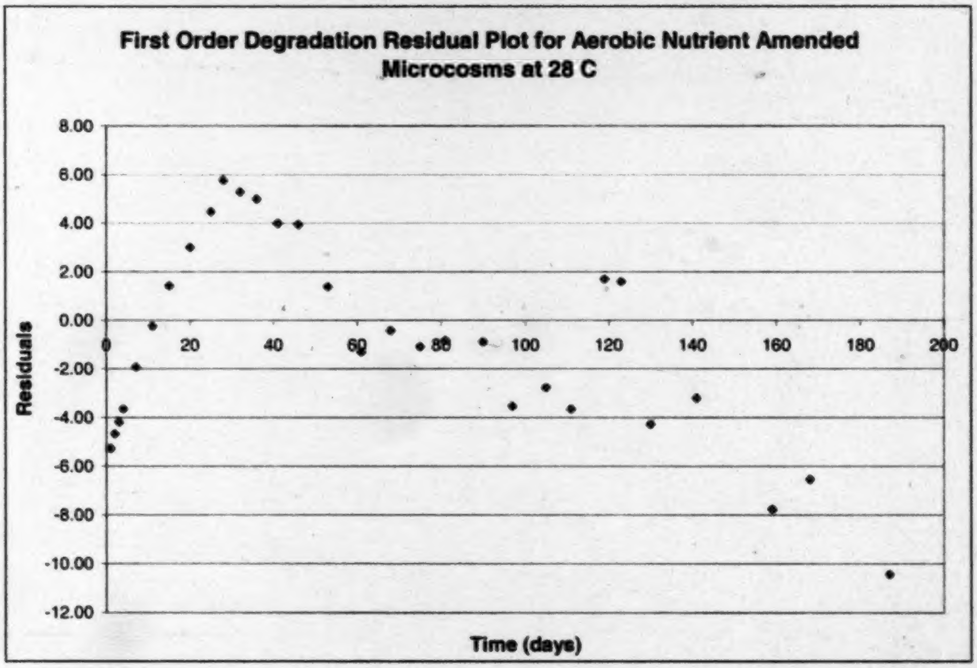


First Order Degradation Residual Plot for Aerobic Unamended Microcosms at 10 C



First Order Degradation Residual Plot for Aerobic Unamended Microcosms at 28 C


First Order Degradation Residual Plot for Aerobic Nutrient Amended Microcosms at 10 C
Residuals
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
-5.00
-10.00
20
40
60
80
100
120
140
160
180
200
Time (days)


First Order Degradation Residual Plot for Aerobic Nutrient Amended Microcosms at 28 C
Residuals
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-10.00
-12.00
0
20
40
60
80
100
120
140
160
180
200
Time (days)